Submit Time: 2/26/2010 23:04:21
From: CN=Rose Allison/OU=DC/O=USEPA/C=US
To: yogesh.p.patel@wv.gov
Cc:
Subject: Conference call

Hi Yogesh Patel, This message is a statement prepared by our Office attorney, Scott Sherlock that works on confidential business information (CBI) about CBI including the handling of CBI. I'm sending this to you in preparation for our scheduled techinical conference call next week, Tuesday, March 2, 2010.

Attached is a copy of the Limited Permission to Disclose Agreement between USEPA and DuPont. By this Agreement, USEPA is authorized to disclose materials and information claimed as confidential business information (CBI) related to two PMN substances to officials of the West Virginia government. In the absence of this Agreement, Federal law places severe limitations on the Agency's ability to share such information.

Please note in particular paragraph 5 of the Agreement which provides that "USEPA/OPPT will inform the participating WVDEP and WVDHHR personnel of the existence of any information claimed as confidential or proprietary by DuPont and treated as confidential by USEPA/OPPT, at the time of disclosure." Also note paragraph 6 which provides that DuPont "makes this limited disclosure with the understanding and belief that WVDEP and WVDHHR will give any information claimed as confidential or proprietary by DuPont and treated as confidential by the USEPA/OPPT all protections to which it is entitled under applicable law."

If you could please acknowledge by email that you and others expected to have access to the CBI and other materials have seen this email, reviewed the Agreement and undersand the terms under which DuPont is authorizing USEPA/OPPT to discuss the related matters with WVDEP and WVDHHR, that would be appreciated. Once this is done, we can initiate all actions (e.g. the conference call) referenced and contemplated under the Agreement.

Concerning the conference call, the EPA participants are scheduled to be Greg Fritz,chemist, Steven Cragg, toxicilogist, Sara Pollack ecotoxicologist, Majd El-Zoobi, chemical engineer (who deals with possible exposures and releases of the new chemical) and me, Rose Allison, program manager. If you could send a proposed agenda and your participants on Monday that would be helpful. Do you want to call me?. I will have to provide you a conference room phone number.

Regards, Rose Allison

P08-508 & 509 limited disclosure.pdf P08-508 & 509 limited disclosure.pdf

Rose Allison For Deliveries
Team Leader **EPA East Building**
New Chemicals Program *1201 Constitution Ave NW
Chemical Control Division (7405M) **Room 4419H**
US EPA **Wash DC 20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

From: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>
Subject: Re: Draft Agenda for meeting with Ms. Seed
Submit Time: 12/16/2009 21:49:00
To: Rose Allison/DC/USEPA/US@EPA

Rose,
Yes that is correct. It must have wrapped when I sent it. Sorry for the confusion.
Kind regards,
Jane
sent from my Blackberry Wireless Handheld

----- Original Message -----
From: Allison.Rose
Sent: 12/16/2009 04:44 PM EST
To: Jane Bradd Andersen
Subject: Re: Draft Agenda for meeting with Ms. Seed

Jane, There are only 2 items on the agenda, right? The third line is connected to the second. Rose

---

Rose Allison
Deliveries
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For
EPA East Building
*1201 Constitution Ave NW
Room 4419H
**Wash DC

From: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>

To: Rose Allison/DC/USEPA/US@EPA

Date: 12/16/2009 12:59 PM

Subject: Draft Agenda for meeting with Ms. Seed

Hello Rose!

I finally have a rough draft for the discussion with Jennifer. Let me know
what you think. Attendee's should be Jim Hoover **PBI / Ex. 4** and I.

Update of 90-day studies in rats and mice with P-08-509
Discussion of species guidance for Chronic Toxicity/carcinogenicity study in the P-08-508/509 Consent Order

Kind regards,

Jane Bradd-Andersen
tel:302-999-2377
fax:302-999-2177
jane-bradd.andersen@usa.dupont.com

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Submit Time: 11/16/2009 21:50:12
From: CN=Rose Allison/OU=DC/O=USEPA/C=US
To: CN=Greg Schweer/OU=DC/O=USEPA/C=US@EPA CN=Scott Sherlock/OU=DC/O=USEPA/C=US@EPA
Cc:
Subject: Fw: Form/Information for cbi authorization for conference call

This is what I got back from the Company. Any reactions? I need to compare it side-by-side with our draft. At first glance, it seems ok to me. There is no CBI in this document, although the Company has asked that since it's a draft document that we limit access so please do not forward. I'll keep a record. They're fine with using the email.

Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

----- Forwarded by Rose Allison/DC/USEPA/US on 11/16/2009 04:43 PM -----

http://www.DuPont.com/corp/email_disclaimer.html

Limited Permission to Disclose Draft 11.12.09.doc Limited Permission to Disclose Draft 11.12.09.doc

Subject: Fw: Protocol Review for P08-509
To: CN=Jennifer Seed/OU=DC/O=USEPA/C=US@EPA
Cc: CN=Rose Allison/OU=DC/O=USEPA/C=US@EPA CN=Bob Morcock/OU=DC/O=USEPA/C=US@EPA CN=Gordon Cash/OU=DC/O=USEPA/C=US@EPA
From: CN=Donald Rodier/OU=DC/O=USEPA/C=US
Submit Time: 12/4/2009 16:52:28

Hi Jennifer,

Please see Rose's note below. I had sent a mouse reproduction/development study (gavage) to Gordon Cash foe SRC review. Rose mentioned that you or one of your staff would be better suited for the task. Please let me know what you would like to do. I am still learning what I am supposed to be doing.

Don

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

----- Forwarded by Donald Rodier/DC/USEPA/US on 12/04/2009 11:47 AM -----

From: Rose Allison/DC/USEPA/US

To: Donald Rodier/DC/USEPA/US@EPA

Date: 12/04/2009 11:33 AM

Subject: Re: Protocol Review for P08-509

Please ask Jennifer. I'd prefer that she is involved so either she or someone on he staff should do it, I think. Rose

Rose Allison
202/564-8970/FAX 202/564-9490

Donald Rodier---12/04/2009 10:56:33 AM---Hi, I will check with Jennifer about the protocols for this test. Is it alright for SRC to start re

From: Donald Rodier/DC/USEPA/US

To: Rose Allison/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA, Jennifer Seed/DC/USEPA/US@EPA

Date: 12/04/2009 10:56 AM

Subject: Re: Protocol Review for P08-509

Hi,

I will check with Jennifer about the protocols for this test. Is it alright for SRC to start reviewing it or do you believe that one of Jennifer's staff should do it?

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

Rose Allison---12/04/2009 10:20:29 AM---Don, Jennifer has been involved in these protocol reviews and in my mind should be consulted. This

From: Rose Allison/DC/USEPA/US

To: Donald Rodier/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA

Date: 12/04/2009 10:20 AM

Subject: Protocol Review for P08-509

Don, Jennifer has been involved in these protocol reviews and in my mind should be consulted. This is a protocol that has specific modifications that we required and we've allowed some deviation based on ORD's more recent work. Rose.

Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

Hi Gordon,

You may have already seen Oscars note about my handling past due PMN cases. I am already getting actions. This email is about a 5e order. We are supposed to review a protocol for an oral (gavage)

reproduction/development study with mice. The PMN is P-08-509 and the DCO number is 5010000683. Would you please have SRC review this protocol. Although the due date is January 5, we need time for a QA/QC and I don't have a clue who will do this, so could you ask SRC to complete it by Dec.23? You can tell I am new at this so if I have omitted anything please let me know.

Don

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

Subject: Re: Fw: Protocol Review for P08-509
To: CN=Rose Allison/OU=DC/O=USEPA/C=US@EPA
Cc: CN=Donald Rodier/OU=DC/O=USEPA/C=US@EPA
From: CN=Bob Morcock/OU=DC/O=USEPA/C=US
Submit Time: 12/14/2009 20:53:38

Yep. Posting the final review memo on the CBI LAN is assumed, but I neglected to mention that as well. Thanks for the reminder.

Rose Allison---12/14/2009 03:47:28 PM---And putting it on the CBI LAN j: drive; PostFocus under the case no. P08-508 and 509? _____

From: Rose Allison/DC/USEPA/US

To: Bob Morcock/DC/USEPA/US@EPA

Cc: Donald Rodier/DC/USEPA/US@EPA

Date: 12/14/2009 03:47 PM

Subject: Re: Fw: Protocol Review for P08-509

And putting it on the CBI LAN j: drive; PostFocus under the case no. P08-508 and 509?

Rose Allison For Deliveries
Senior Specialist **EPA East Building**
New Chemicals Program *1201 Constitution Ave NW
Chemical Control Division (7405M) **Room 4419H**
US EPA **Wash DC 20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

Bob Morcock---12/14/2009 02:39:33 PM---Jennifer: I would address the memo to Rose. Also we need a signed hardcopy for RAD and CBIC files.

From: Bob Morcock/DC/USEPA/US

To: Jennifer Seed/DC/USEPA/US@EPA

Cc: Donald Rodier/DC/USEPA/US@EPA, Rose Allison/DC/USEPA/US@EPA

Date: 12/14/2009 02:39 PM

Subject: Re: Fw: Protocol Review for P08-509

Jennifer:

I would address the memo to Rose. Also we need a signed hardcopy for RAD and CBIC files.

Just route it to one of our DCOs, i.e., Vivian Hart, Eileen White, or Carolyn Whitaker, who can make sure it gets put into the proper hopper for distribution.

Bob

Rose Allison---12/14/2009 10:08:07 AM---Bob and Don, Do you have any input about who the memo should go to? It can go to me or one of you.

From: Rose Allison/DC/USEPA/US

To: Bob Morcock/DC/USEPA/US@EPA, Donald Rodier/DC/USEPA/US@EPA

Date: 12/14/2009 10:08 AM

Subject: Fw: Protocol Review for P08-509

Bob and Don, Do you have any input about who the memo should go to? It can go to me or one of you. Just let me know. Rose

Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

----- Forwarded by Rose Allison/DC/USEPA/US on 12/14/2009 10:06 AM -----

From: Jennifer Seed/DC/USEPA/US

To: Rose Allison/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA, Donald Rodier/DC/USEPA/US@EPA, Greg Schweer/DC/USEPA/US@EPA

Date: 12/11/2009 12:23 PM

Subject: Re: Fw: Protocol Review for P08-509

You can give the company the thumbs up. Should the memo be addressed to you?

Jennifer Seed, PhD
Deputy Director
Risk Assessment Division, OPPT
202-564-7634
seed.jennifer@epa.gov

Rose Allison---12/10/2009 12:18:51 PM---Yes, a formal memo is good. For P06-462 et al you gave it to Bob Morcock and it was added to the CB

From: Rose Allison/DC/USEPA/US

To: Jennifer Seed/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA, Donald Rodier/DC/USEPA/US@EPA, Greg Schweer/DC/USEPA/US@EPA

Date: 12/10/2009 12:18 PM

Subject: Re: Fw: Protocol Review for P08-509

Yes, a formal memo is good. For P06-462 et al you gave it to Bob Morcock and it was added to the CBI LAN under the PMN no. in the POSTFOCUS file on the J; drive.

Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

Jennifer Seed---12/09/2009 07:36:36 PM---Sure why not! Do I need to write one of those formal memos? If so who do I address it to and who d

From: Jennifer Seed/DC/USEPA/US

To: Rose Allison/DC/USEPA/US@EPA, Donald Rodier/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA

Date: 12/09/2009 07:36 PM

Subject: Re: Fw: Protocol Review for P08-509

Sure why not! Do I need to write one of those formal memos? If so who do I address it to and who do I give it to - I do not have CBI lan rights
Rose Allison

----- Original Message -----
From: Rose Allison
Sent: 12/09/2009 04:56 PM EST
To: Jennifer Seed; Donald Rodier
Cc: Bob Morcock
Subject: Re: Fw: Protocol Review for P08-509

Thanks Jennifer. I was speaking with the company contact and they told me that if it could be reviewed this month that they'd get some break on getting it started, so I was wondering if there was any possibility for review by the week of Dec. 21? I thought I'd ask. Rose

Rose Allison
Senior Specialist

For Deliveries
EPA East Building

New Chemicals Program *1201 Constitution Ave NW
Chemical Control Division (7405M) **Room 4419H**
US EPA **Wash DC 20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

Yes, do not send this to SRC. If someone can get me the protocol we can get it looked at.

Jennifer Seed, PhD
Deputy Director
Risk Assessment Division, OPPT
202-564-7634
seed.jennifer@epa.gov

Donald Rodier---12/04/2009 11:52:28 AM---Hi Jennifer, Please see Rose's note below. I had sent a mouse reproduction/development study (gavag

From: Donald Rodier/DC/USEPA/US

To: Jennifer Seed/DC/USEPA/US@EPA

Cc: Rose Allison/DC/USEPA/US@EPA, Bob Morcock/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA

Date: 12/04/2009 11:52 AM

Subject: Fw: Protocol Review for P08-509

Hi Jennifer,

Please see Rose's note below. I had sent a mouse reproduction/development study (gavage) to Gordon Cash foe SRC review. Rose mentioned that you or one of your staff would be better suited for the task. Please let me know what you would like to do. I am still learning what I am supposed to be doing.

Don

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

----- Forwarded by Donald Rodier/DC/USEPA/US on 12/04/2009 11:47 AM -----

From: Rose Allison/DC/USEPA/US

To: Donald Rodier/DC/USEPA/US@EPA

Date: 12/04/2009 11:33 AM

Subject: Re: Protocol Review for P08-509

Please ask Jennifer. I'd prefer that she is involved so either she or someone on he staff should do it, I think. Rose

Rose Allison
202/564-8970/FAX 202/564-9490

Donald Rodier---12/04/2009 10:56:33 AM---Hi, I will check with Jennifer about the protocols for this test. Is it alright for SRC to start re

From: Donald Rodier/DC/USEPA/US

To: Rose Allison/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA, Jennifer Seed/DC/USEPA/US@EPA

Date: 12/04/2009 10:56 AM

Subject: Re: Protocol Review for P08-509

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Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

Rose Allison---12/04/2009 10:20:29 AM---Don, Jennifer has been involved in these protocol reviews and in my mind should be consulted. This

From: Rose Allison/DC/USEPA/US

To: Donald Rodier/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA

Date: 12/04/2009 10:20 AM

Subject: Protocol Review for P08-509

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Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

Hi Gordon,

You may have already seen Oscars note about my handling past due PMN cases. I am already getting actions. This email is about a 5e order. We are supposed to review a protocol for an oral (gavage) reproduction/development study with mice. The PMN is P-08-509 and the DCO number is 5010000683. Would you please have SRC review this protocol. Although the due date is January 5, we need time for a QA/QC and I don't have a clue who will do this, so could you ask SRC to complete it by Dec.23? You can tell I am new at this so if I have omitted anything please let me know.

Don

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

From: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>
To: Rose Allison/DC/USEPA/US@EPA
Subject: Modified 1-generation Reproduction Study [OPPTS 870.3550]
Submit Time: 6/8/2010 14:29:16

Dear Rose:

As a follow up to our conversation from Tuesday, June 1, 2010..... I am submitting for Agency approval modifications to the protocol for Modified One-Generation Reproduction Study in Mice. This protocol was initially approved by the Agency on November 2009. The Agency provided approval for Amendment 4 on April 28, 2010 and Amendment 5 on May 3, 2010 [see attached emails]. DuPont recognized other changes have occurred to the protocol subsequent to the initial Agency approval.

With this email I am requesting Agency approval for Amendments 1 through 3 to the protocol for Modified One-Generation Reproduction Study in Mice.

The following document is a copy of the protocol where the changes are embedded and highlighted using "track changes" tool for Microsoft Word.

The following is a copy of the protocol and changes as per the process employed by DuPont to satisfy GLP requirements.

Kind regards,

Jane Bradd-Andersen
tel:302-999-2377
fax:302-999-2177
jane-bradd.andersen@usa.dupont.com

********** ***AMENDMENT 4 APPROVAL*** **********

Allison.Rose@epamail.epa.gov

04/28/2010 01:35 PM

To Jane Bradd Andersen/AE/DuPont@DuPont

cc

Subject Re: April 7, 2010 Meeting request item

Hi Jane, Yes, OPPT/EPA approves the amendment. Rose

Rose Allison
Team Leader
New Chemicals Program
Chemical Control Division (7405M)

For Deliveries
EPA East Building
*1201 Constitution Ave NW *
Room 4419G

US EPA **Wash DC
20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

From: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>

To: Rose Allison/DC/USEPA/US@EPA

Date: 04/26/2010 06:18 PM

Subject: April 7, 2010 Meeting request item

Hi Rose,

At the April 7, 2010 meeting with representatives from DuPont and the Agency, it was suggested that DuPont include plasma sample collection and analysis as part of the Modified one-Generation Reproduction study [OECD 421, modified] which was approved by the Agency on January 29, 2010. This subject was again discussed during the subsequent conference call on April 22, 2010 between representatives from DuPont and the Agency.

Can you please confirm Agency approval with the proposed amendment to the study? I have attached a copy of the proposal submitted to the Agency on April 14, 2010.

Kind regards,

Jane Bradd-Andersen
tel:302-999-2377
fax:302-999-2177
jane-bradd.andersen@usa.dupont.com

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[attachment "189225 Draft Amendment 4 041210.doc" deleted by Rose Allison/DC/USEPA/US]

**********AMENDMENT 5 APPROVAL **********

Seed.Jennifer@epamail.epa.gov

05/03/2010 09:10 AM

To James R Hoover/AE/DuPont@DuPont

cc Schweer.Greg@epamail.epa.gov

Subject Re: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Jim,

Sorry about the confusion over this. The protocol is fine. I hope you get this message in time.

Jennifer

Jennifer Seed, PhD
Deputy Director
Risk Assessment Division, OPPT
202-564-7634
seed.jennifer@epa.gov

|---------->
| From: |
|---------->
>----------|
|James R Hoover <James.R.Hoover@USA.dupont.com>
|
>----------|
|---------->
| To: |
|---------->
>----------|
|Jennifer Seed/DC/USEPA/US@EPA

|
>----------|
|---------->
| Date: |
|---------->
>----------|
|04/30/2010 04:16 PM |
>----------|
|---------->
| Subject: |
|---------->
>----------|
|Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -
|
>----------|

Jennifer....Greg Schweer directed me to Jim Allwood, and Jim told me to foward this EMail and give you a quick call, so I will call in a minute. May apologies for this,best rgds, Jim

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 04:14 PM -----

James R
Hoover/AE/DuPont

To
04/30/2010 03:57 PM schweer.greg@epa.gov
cc

Subject
Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Hi Greg....My personal apologies for this very late discovery, and even later communication to Rose and to you, on this issue. This is clearly our mistake, and I take full responsibility for it.

I called Rose to give her a heads-up on the enclosed EMails (below). I now understand from Rose's Voicemail that she is out today.

For us to proceed as indicated, I think we would need a "non-objection" Email from EPA relative to this EMail before 6:00am this coming Monday

morning (May 3rd).

The details are show below.

Any advice would be much appreciated. We fully realize this may be difficult to impossible, but I wanted to make fully sure that what ever we do is totally right.

Again, my apologies.

Very best regards, Jim

Jim Hoover, FPS Global Regulatory Manager

DuPONT DCF/FPS
CRP 702, Room 2116
Wilmington, DE 19880

BBerry: Personal Phone / Ex. 6

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 03:40 PM -----

James R Hoover/AE/DuPont
04/30/2010 03:11 PM

To: Allison.Rose@epamail.epa.gov

cc: Gary W Jepson/AE/DuPont@DuPont, Steven R Frame/AE/DuPont@DuPont, Susan M Munley/AE/DuPont@DuPont, Jane Bradd Andersen/AE/DuPont@DuPont

Subject: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Hi Rose...Jane is away on vacation, and out of communication range.

My personal, and DuPont company, apologies for this late and urgent 'non-objection' request consideration, but our GenX Toxicity Team has just realized that we overlooked a CRITICAL study design issue in the 18405-1937 Mouse Study Blood Collection Amendment just approved by EPA.

The details are outlined in the Notes from Randy Frame and Sue Munley,

shown below.

Given the circumstances and timing, what options do we have to proceed, with EPA agreement, for what Randy and Sue recommend (i.e. a non-objection to proceed).

We fully realize this may be difficult to impossible, but I wanted to make fully sure that what ever we do is totally right.

Many thx for your advice...best regards, Jim

Jim Hoover, FPS Global Regulatory Manager

DuPONT DCF/FPS
CRP 702, Room 2116
Wilmington, DE 19880

BBerry Personal Phone / Ex. 6

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 02:50 PM -----

Steven R
Frame/AE/DuPont

04/30/2010 11:46 AM

To
James R Hoover/AE/DuPont@DuPont, Jane Bradd Andersen/AE/DuPont@DuPont

cc
Gary W Jepson/AE/DuPont@DuPont, Susan M Munley/AE/DuPont@DuPont

Subject
URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Jim, Jane,

Please see Sue's note below. In order to get any useful results from the blood collection on adult females in the mouse study, we need to administer a dose on the day of sacrifice (in the current protocol, the last dose is the day before sacrifice). Without a day-of-sacrifice dosing, the blood data from the moms will be of little value. Therefore, it is near certain the EPA would concur with this minor change in procedure since they suggested the blood collection in the first place, and they undoubtedly want the most useful information. Further, this would have no effect on the study results since the animals are sacrificed very soon after the extra dose. Nevertheless, we

will need your OK, and EPA's OK to proceed, and we must have this OK before Monday due to the stage of the test we are in. The new amendment could be to the EPA on Monday or soon thereafter.

Randy
----- Forwarded by Steven R Frame/AE/DuPont on 04/30/2010 11:38 AM -----

Susan M
Munley/AE/DuPont

To
04/30/2010 11:37 AM Steven R Frame/AE/DuPont@DuPont, Gary W Jepson/AE/DuPont@DuPont
cc

Subject
18405-1037 mouse study blood collection amendment - URGENT ISSUE

While preparing to execute the blood collection for plasma TK as dictated by the recently-approved protocol amendment 4, we realized that we overlooked a CRITICAL study design issue.

As per protocol, adult animals are scheduled to be dosed through one day PRIOR to scheduled euthanasia.

Based on existing data, blood collection scheduled for two hours following the last dose is the optimal and most meaningful time for collection.

We cannot obtain enough volume for this work without making the bleed a terminal bleed.

Therefore, we need to write another amendment (draft attached below) to specify that animals will be administered a single additional dose on the morning of scheduled euthanasia and then euthanized two hours following that dose.

I am writing to seek non-objection to proceed with this beginning this coming Monday morning, May 3.

The first F0 females to reach PND 21 are scheduled to have their litters weaned and be subsequently euthanized this coming Monday morning.

As these procedures will clarify and improve upon the data dictated by the previously approved amendment 4, please let me know if we can proceed with approving this work to begin on Monday.

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Francais Deutsch Italiano Espanol Portugues Japanese Chinese Korean

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(See attached file: 189225 Draft Amendment 5 043010.doc)

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189225 Draft Amendment 5 043010.doc 18405-1037 complete protocol smm 19 may 2010.pdf 18405-1037 protocol with amendments as tracked changes smm june 3, 2010.pdf

PROTOCOL

AN ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY OF H-28548 IN MICE

(U.S. EPA OPPTS 870.3550 and OECD Guideline 421)

Submitted To:

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898

DuPont Work Request Number: 18405
DuPont Service Code: 1037
DuPont Study Number: 18405-1037

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

ED_002003A_00062142-00001

1 OBJECTIVE:

To provide preliminary information on the potential adverse effects of the test substance on male and female reproduction within the scope of a screening study. This will encompass gonadal function, mating behavior, conception, parturition and lactation of the F_0 generation and the development of offspring from conception through day 40 of postnatal life.

This study is subject to the applicable regulations of the Organisation for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Guideline 421, Reproduction/Development Toxicity Screening Test, July 27, 1995, and the United States Environmental Protection Agency (EPA) Health Effects Test Guidelines OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test, July 2000 and will be conducted in accordance with the EPA/TSCA and FIFRA (40 CFR Part 792 and 40 CFR Part 160) and the OECD Principles of Good Laboratory Practice.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Study Representative:

Susan M. Munley, MA
Research Toxicologist
Developmental, Reproductive and Neurobehavioral Toxicology
DuPont Haskell Laboratory for Health and Environmental Sciences
1090 Elkton Rd., PO Box 50
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2.2 Principal Investigator, Pathology

Greg P. Sykes, VMD, DACVP, DACLAM, DABT
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2.3 WIL Study Director:

Tammye L. Edwards, BS, LAT
Staff Toxicologist, Developmental and Reproductive Toxicology
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2.4 WIL Departmental Responsibilities:

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Donald G. Stump, PhD, DABT
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Reproductive Toxicology

George A. Parker, DVM, PhD, DACVP, DABT
Director, Pathology

Melissa J. Beck, PhD
Assistant Director, Neurosciences

Daniel W. Sved, PhD
Director, Metabolism and Analytical Chemistry

Walter R. Miller, BS, DVM
Clinical Veterinarian,
Head of Surgery and Experimental Medicine

Ronald E. Wilson, BS
Director, Informational Systems

Carol A. Kopp, BS, LAT
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

Bennett J. Varsho, MPH, DABT
Operations Manager, Developmental and
Reproductive Toxicology and the Formulations Laboratory

Robert A. Wally, BS, RAC
Manager, Reporting and Regulatory
Technical Services

3 STUDY SCHEDULE:

Proposed Experimental Starting (Animal Receipt) Date:	5 January 2010
Proposed Experimental Start (First Day of Dosing) Date:	14 January 2010
Proposed Experimental Completion/Termination Date:	4 June 2010
Proposed Audited Report Date:	To be determined

4 TEST SUBSTANCE DATA:

4.1 Test Substance Shipment:

Test substance and applicable documentation, including a Certificate of Analysis, will be shipped under Sponsor's responsibility to:

Formulations Laboratory (WIL-189225; Tammye Edwards)
Attn: Larry Blessing
WIL Research Laboratories, LLC
1407 George Road
Ashland, Ohio 44805-8946

4.2 Identification:

H-28548 or HFPO Dimer Acid Ammonium Salt

4.3 **Haskell Test Substance Number:**

H-28548

4.4 **Lot Number:**

E109540-44A

4.5 **Expiration/Retest Date:**

13 June 2011

4.6 **Purity:**

84%

4.7 **Storage Conditions:**

Controlled room temperature and humidity (approximately 18° to 24°C and 20% to 70% relative humidity)

4.8 **Stability:**

The analysis was performed by the Sponsor and documented on the Certificate of Analysis.

4.9 **Physical Description:**

To be documented by WIL Research Laboratories, LLC.

4.10 **Reserve Samples:**

Reserve samples of the test substance will be taken in accordance with WIL Standard Operating Procedures and stored in the Archives at WIL Research Laboratories, LLC indefinitely, unless otherwise specified.

4.11 **Personnel Safety Data:**

See the Material Safety Data Sheet (MSDS) provided by the Sponsor.

4.12 **Test Substance Disposition:**

With the exception of the reserve sample for each batch of test substance, which will be archived as described, all neat test substance remaining at completion of the in-life phase of the study will be kept for subsequent studies.

5 TEST SYSTEM:

5.1 Species:

Mouse

5.2 Strain:

Charles River Crl:CD1(ICR)

5.3 Source:

Males: Charles River Laboratories, Inc., Raleigh, NC
Females: Charles River Laboratories, Inc., Kingston, NY

5.4 Number on Study:

100 males and 100 females (minimum of 120 males and 120 females purchased; males and females will be ordered from separate facilities to ensure the avoidance of sibling mating). Animals not assigned to study will be transferred to the stock animal colony or will be euthanized by carbon dioxide inhalation and the carcasses discarded.

The number of animals used on this study is consistent with OPPTS and OECD guidelines for reproduction/developmental toxicity screening studies.

5.5 Body Weight Range:

A minimum of 20 grams at randomization.

5.6 Approximate Age:

42-63 days old at randomization.

5.7 Identification System:

Each mouse will be uniquely identified by tattoo markings applied to the tail. Individual cage cards will be affixed to each cage and will display the animal number, group number, study number, dosage level and sex of the animal.

5.8 Justification for Selection:

This species and strain of animal is recognized as appropriate for reproduction studies. WIL Research Laboratories, LLC has reproductive historical control data in the Crl:CD1(ICR) mouse. This animal model has been proven to be susceptible to the effects of reproductive toxicants.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed, 2-3 per cage, for at least 3 days following receipt. Thereafter, the mice will be housed individually. The F_0 males and females will be individually housed in solid bottom cages (plastic maternity cages) containing ground corncob nesting material (Bed-O' Cobs®) in an environmentally controlled room during the quarantine period and throughout the entire study until euthanasia. All F_1 offspring not euthanized at weaning will be housed by litter in the plastic cages with nesting material until postnatal day (PND) 28. F_1 offspring not selected for the maturation phase will be necropsied on PND 21. On PND 28, F_1 offspring will be individually housed in solid bottom cages (plastic maternity cages) containing ground corncob nesting material (Bed-O' Cobs®). The cages will be subject to routine cleaning at a frequency consistent with maintaining good animal health and WIL Standard Operating Procedures. The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain temperature at 71 ± 5°F (22 ± 3°C) and relative humidity at 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Reverse osmosis-purified water will be available *ad libitum*. Filters servicing the automatic watering system are changed regularly according to WIL Standard Operating Procedures. The municipal water supplying the laboratory is analyzed according to WIL Standard Operating Procedures on a routine basis to ensure that contaminants are not present in concentrations that would be expected to affect the outcome of the study.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 will be offered *ad libitum* during the study. Periodic analyses of the certified feed are performed by the manufacturer to ensure that heavy metals and pesticides are not present at concentrations that would be expected to affect the outcome of the study. Results of the analyses are provided to WIL Research Laboratories,

LLC by the manufacturer. Feeders will be changed and sanitized once per week.

6.5 Enrichment:

All animals will be offered Nestlets™ for enrichment that will be replaced as needed.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Quarantine:

Each animal will be inspected by a qualified technician upon receipt. Mice judged to be in good health and suitable as test animals will be immediately placed in quarantine for a minimum of 9 days. All mice will be initially weighed, permanently identified by tattoo markings applied to the tail and receive a clinical observation. During the quarantine period, each mouse will be observed twice daily for changes in general appearance and behavior. Prior to the start of the in-life phase, those animals judged to be suitable test subjects will be identified and receive a detailed physical examination.

7.2 Randomization:

At the conclusion of the quarantine period, animals judged to be suitable test subjects and meeting acceptable body weight requirements, will be assigned at random using a computer program. At that time, the animal numbers and corresponding body weights will be entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers and individual group assignments will be generated based on body weight stratification into a block design. Animals will then be arranged into the groups according to the printout. The control group and three test item groups will consist of 20 males and 20 females each.

Any animal assigned to the study that is found dead, euthanized *in extremis* or exhibits abnormal clinical signs, reduced food consumption or body weight losses prior to the start of dosing may be replaced by an animal of appropriate age when possible. Replacement animals will be arbitrarily assigned (not computer randomized) to the study based on comparable body weights (if possible) with respect to the animal that was replaced.

7.3 Route and Rationale of Test Item Administration:

The route of administration will be oral (gavage). Historically, this route has been used extensively for studies of this nature. Appropriately sized flexible,

Teflon®-shafted, stainless steel ball-tipped dosing cannulae will be used for the oral administration by gavage.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The dose levels proposed for the current study are 0, 0.1, 0.5, and 5 mg/kg/day and are based on previous and ongoing general toxicity studies in mice. These levels are currently being tested in an ongoing (in-life dosing phase complete) subchronic toxicity 90-day gavage study (DuPont-18405-1307). The doses for the 90-day gavage study were based on results from a previous 28-day gavage study (DuPont-24459) in which doses of 0, 0.1, 3, and 30 mg/kg/day were tested.

The following table presents the study group arrangement.

Group Number	Test Item	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Male	Female
1	Vehicle Control[b]	0	0	10	25	25
2	H-28548	0.1	0.01	10	25	25
3	H-28548	0.5	0.05	10	25	25
4	H-28548	5	0.5	10	25	25

[a] Dosage levels will be corrected for the purity of 84%.
[b] Deionized Water

7.4.2 Vehicle Control Item:

Deionized Water

7.4.3 F_0 Treatment Regimen:

The test and control items will be administered once daily at approximately the same time each day as follows:

7.4.3.1 Males:

F_0 males will be dosed for a minimum of 70 days prior to mating and continuing until the day prior to the scheduled euthanasia.

7.4.3.2 Females:

F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 21 for females that deliver. For females

that do not have positive signs of mating or delivery, dosing will continue until one day prior to euthanasia.

7.4.3.3 F_1 Males and Females:

F_1 males and females will be dosed beginning in PND 21 until one day prior to euthanasia.

7.4.4 Adjustment of Dosages:

Individual dosages will be calculated based on the most recent body weight to provide the proper mg/kg/day dosage.

7.5 Preparation and Analysis of Test Item Formulations:

7.5.1 Method and Frequency of Preparation:

Based on the physical characteristics of the test substance, appropriate methods will be used to ensure the best possible formulations of the test substance in the vehicle. Dosing formulations will be stored refrigerated (2-8°C) for a maximum of 12 days. The Study Director or designee will visually inspect the formulations prior to the initiation of dosing. This visual inspection will be performed to ensure that the formulations are visibly homogeneous and acceptable for dosing. Any special procedures required for formulation will be documented according to Good Laboratory Practices and presented in the final report of this study. Test substance formulations will be prepared approximately weekly and divided into aliquots for daily dispensation. The test substance and vehicle formulations will be stirred continuously during dosing.

7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Substance Formulations:

Stability and resuspension homogeneity were established on a previous study (Haas, Draft; WIL-189216). Test substance formulations were stable and 12 days of room temperature storage or refrigerated storage (2-8°C) at concentrations of 0.01 mg/mL and 100 mg/mL and homogenous following resuspension after 12 days of refrigerated storage (2-8°C). Stability and resuspension homogeneity will not be conducted on this study.

Homogeneity and concentration will be conducted on the first formulations prepared for dosing. Four 1-mL samples will be collected from the top, middle and bottom of the test substance formulations from the low and high dose groups and the samples analyzed to assess the

homogeneity of the test substance in the mixtures; the middle strata will serve as the measure of test substance concentration. Four 1-mL samples will be taken from the middle on the control and the mid-dose groups and analyzed for concentration of the test substance.

Concentration will be assessed on Week 4, 8, 12, 16 and 19 formulations prepared for dosing. Four 1-mL samples will be collected from the middle of each test substance formulation and the control group and analyzed for test substance content.

7.5.3 Sample Analysis:

Samples will be transferred to the Analytical Chemistry Department at WIL Research Laboratories, LLC for analysis. Analyses of test article formulations will be performed using a method developed and validated by WIL Research Laboratories, LLC. Initially, two of each set of four replicate, 1-mL samples will be analyzed; the remaining two 1-mL samples will be stored frozen (approximately -20°C) at WIL and will function as back-up samples. Back-up samples will be analyzed if requested by the Sponsor or Study Director or may be discarded following results that are within specifications and approval of the Study Director.

7.6 F_0 Breeding:

After a minimum of 70 days for males and 14 days of exposure for females, of exposure, one female will be cohabitated with one male mouse of the same treatment group, avoiding sibling mating, in a plastic cage for mating. Detection of mating will be confirmed by evidence of sperm in the vaginal lavage. After confirmation of mating, the female will be returned to an individual plastic cage and the day will be designated as day 0 of gestation.

A maximum of 14 days will be allowed for mating. After 14 days of mating, any females who have not shown evidence of breeding will be placed in a plastic cage containing nesting material.

7.7 F_0 Parturition and Lactation and F_1 Litters:

The day parturition is initiated will be designated as day 0 of lactation. Any difficulties at the time of parturition will be recorded. When parturition is judged to be complete, the sex of each pup will be determined, pups will be examined for gross malformations and the number of stillbirths and live pups will be recorded. Any changes or abnormalities in nesting and nursing behavior will be recorded. The dam and litter will remain together until postnatal day (PND) 21.

7.8 Identification of F_1 Litters:

Upon completion of delivery, all pups will be individually identified by tattoo markings applied to the digits. To reduce variability among the litters, on PND 4, eight pups of equal sex distribution (if possible) from each litter will be randomly selected. For litters consisting of fewer than eight pups, adjustments for litter sizes will not be performed. Following selection, the non-selected PND 4 pups will be euthanized by an intraperitoneal injection of sodium pentobarbital and discarded.

7.9 General Observations During the Experimental Period:

7.9.1 Parental Appearance and Behavior:

Each parental mouse (F_0) will be observed twice daily for moribundity and mortality, once in the morning and once in the afternoon. A detailed physical examination will be conducted weekly. Mortality and all signs of overt toxicity will be recorded on the day observed. The observations shall include, but are not limited to, evaluations for changes in appearance of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior. During the period of expected parturition, the dams will be observed twice daily for dystocia, prolonged labor, delayed labor or other difficulties at parturition. All animals will also be observed on the day of necropsy and findings will be recorded.

During the treatment period, each animal will be observed at approximately 1-2 hours following each dose administration for findings that are potentially related to treatment of that might change before the next scheduled observation. Additional post dosing observation periods may be necessary and will be documented in the study records.

7.9.2 Parental Body Weights:

All animals will have a final body weight recorded on the day of euthanasia.

7.9.2.1 Males:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until euthanasia.

7.9.2.2 Females:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until evidence of copulation is observed and on gestation days 0, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.

For females with no evidence of mating, individual body weights will continue to be recorded on a weekly basis until euthanasia.

7.9.3 Parental Food Consumption*:

Individual food consumption will not be recorded during the breeding period because the animals are cohabitated at that time.

7.9.3.1 Males:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until euthanasia.

7.9.3.2 Females:

Recorded individually on a weekly basis beginning on the first day of dose administration, until the start of the mating period. Individual food consumption will be recorded on the day evidence of copulation is observed (GD 0) and on gestation days 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.

For females with no evidence of mating, individual food consumption will continue to be recorded on a weekly basis following the end of the mating period until euthanasia.

7.9.4 Examination of Offspring:

7.9.4.1 Appearance and Behavior:

All pups will be observed daily for general appearance and behavior and survival during lactation. A detailed physical examination will be recorded for each pup on PND 1, 4, 7, 14 and 21. Any abnormalities in nesting and nursing behavior will be recorded. The pups will be sexed on PND 0, 4, 14 and 21.

7.9.4.2 Body Weights:

Each pup will be weighed on PND 1, 4, 7, 14 and 21.

7.9.5 Pup Deaths:

7.9.5.1 Pups 0 to 4 Days of Age:

Moribund pups will be euthanized by an intraperitoneal injection of sodium pentobarbital. Stillborn pups, pups found dead between birth and PND 4, and any pups that are euthanized *in extremis* will be dissected (including the heart and the brain examined by a mid-coronal slice) by a technique described by Stuckhardt and Poppe (Stuckhardt and Poppe, 1984). If a skeletal anomaly is suspected, the pups will be eviscerated, cleared and stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined. Representative specimens with malformations may be preserved in 10% neutral buffered formalin at the discretion of the study director.

7.9.5.2 Pups 5 Days of Age to Weaning:

Moribund pups will be euthanized by an intraperitoneal injection of sodium pentobarbital (prior to PND 11) or by carbon dioxide inhalation. A gross necropsy will be performed on pups found dead or euthanized *in extremis*, and gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin. If a skeletal anomaly is suspected, the pups will be eviscerated, cleared and stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined.

7.10 Selection of F_1 Generation and Termination of PND 21 Nonselected Pups:

One male and one female pup per litter will be selected for the F_1 generation on or prior to PND 21. Only pups not expected to survive due to notable physical limitations will not be available for selection. A detailed evaluation of each pup excluded from selection will be recorded.

All PND 21 pups not selected for the F_1 generation will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

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7.11 Euthanasia of F_0 Generation:

7.11.1 Females:

7.11.1.1 Females Which Deliver:

On lactation day 21, all F_0 females that delivered will be euthanized by carbon dioxide inhalation. A gross examination will be performed and tissues preserved as described in Section 8.1. The number of former implantation sites will be recorded. Organ weights will be collected and tissues preserved as described in Section 8.2.

7.11.1.2 Females Which Fail to Deliver:

On post-mating day 25 (females with evidence of copulation) or post-cohabitation day 25 (females without evidence of copulation), the F_0 females which fail to deliver will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed and tissues will be preserved as described in Section 8.1. Organ weights will be collected as described in Section 8.2 with the exception of any ammonium sulfide stained uterus, which will be discarded. Uteri which appear nongravid by macroscopic examination will be opened and placed in a 10% ammonium sulfide solution (Salewski, 1964) for detection of early implantation loss.

7.11.1.3 Females with Total Litter Loss:

Females with total litter loss will be euthanized by carbon dioxide inhalation on the same day. The number of former implantation sites will be recorded and the number of corpora lutea (if litter loss occurs on or before PND 4) will be recorded. A gross necropsy examination will be performed and tissues preserved as described in Section 8.1. Organ weights will be collected as described in Section 8.2.

7.11.1.4 F_0 Deaths and Animals Euthanized *in Extremis*:

Females not surviving until the scheduled euthanasia will have a gross necropsy examination performed and tissues preserved as described in Section 8.1. Animals not expected to survive to the next observation period (moribund) will be euthanized by carbon dioxide inhalation and have a gross necropsy examination performed and tissues preserved as described in Section 8.1.

Organ weights will not be collected from found dead or euthanized *in extremis* females. The number and location of implantation sites or scars will be recorded for females dying or euthanized during gestation and lactation. The number of corpora lutea will be recorded for females dying or euthanized during gestation and up to and including lactation day 4. Uteri which appear nongravid by macroscopic examination will be opened and placed in a 10% ammonium sulfide solution (Salewski, 1964) for detection of early implantation loss.

Viable fetuses will be euthanized by an intrathoracic injection of sodium pentobarbital. Recognizable fetuses will be examined externally for gross abnormalities. Representative specimens with malformations may be preserved in 10% neutral-buffered formalin, at the discretion of the study director. For females found dead or euthanized *in extremis* during lactation, all pups will be examined externally and subjected to a necropsy examination according to Section 7.9.5.

7.11.2 Males:

Following completion of the mating period, all F_0 males will be euthanized by carbon dioxide inhalation and subjected to a gross necropsy and tissue preservation as described in Section 8.1. Organ weights will be collected as described in Section 8.2.

Males not surviving until the scheduled euthanasia will be subjected to a gross necropsy and tissue preservation as described in Section 8.1. Any males not expected to survive to the next observation period (moribund) will be euthanized by carbon dioxide inhalation and also necropsied and have tissues preserved as described in Section 8.1. Organ weights will not be collected.

7.12 F_1 Generation General Observations During The Experimental Period:

7.12.1 F_1 Clinical Observations:

Following weaning and selection, the mice will be observed twice daily for moribundity and mortality, once in the morning and once in the afternoon. Clinical observations will be recorded daily. Mortality and all signs of overt toxicity will be recorded on the day observed. The observations shall include, but are not limited to, evaluation for changes in appearance of the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system function,

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somatomotor activity and behavior patterns. All animals will also be observed on the day of necropsy and any findings will be recorded.

During the treatment period, each animal will be observed at approximately 1-2 hours following each dose administration for findings that are potentially related to treatment of that might change before the next scheduled observation. Additional post dosing observation periods may be necessary and will be documented in the study records.

7.12.2 F_1 Body Weights and Food Consumption:

F_1 males and females will be have a body weight recorded approximately weekly, beginning with the start of test diet administration until euthanasia (PND 21, 28, 35 and 40). All animals will have a final body weight recorded on the day of euthanasia.

F_1 males and females will have food consumption recorded individually on an approximately weekly basis beginning on PND 28 until euthanasia (PND 28, 35 and 40). Food consumption will not be collected from PND 21 to PND 28 during group housing for the F_1 males and females.

7.13 F_1 Postweaning Developmental Landmarks:

Offspring selected for the F_1 generation will be evaluated for attainment of the following landmarks of sexual maturity:

7.13.1 Balanopreputial Separation:

Each male pup will be observed for balanopreputial separation beginning on PND 25 as described by Korenbrot *et al.* (Korenbrot 1977). Examination of the males will continue daily until balanopreputial separation is present. The body weight of each male will be recorded on the day of attainment of balanopreputial separation.

7.13.2 Vaginal Patency:

Each female pup will be observed for vaginal patency beginning on PND 21 (only those selected for the F_1 generation) as described by Adams *et al.* (Adams 1985). Examination of the females will continue daily until vaginal patency is present. The body weight of each female will be recorded on the day of attainment of vaginal patency.

7.14 Euthanasia of F_1 Generation:

7.14.1 Scheduled Necropsy

On PND 40, all F_1 animals will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

7.14.2 Unscheduled Deaths or Animals Euthanized *in Extremis*

Any F_1 animals not surviving until the scheduled euthanasia or not expected to survive to the next observation period (euthanized by carbon dioxide inhalation) will be necropsied. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

8 ANATOMIC PATHOLOGY:

8.1 Macroscopic Examination:

A complete necropsy will be conducted on all F_0 parental animals dying spontaneously, euthanized *in extremis* (by carbon dioxide inhalation) or at termination. This will include examination of the external surface, all orifices, the cranial cavity, the external surface of the brain and the thoracic, abdominal and pelvic cavities including viscera. For F_0 females, the number of former implantation sites will be recorded.

At the time of necropsy, the following tissues and organs will be collected and placed in 10% neutral-buffered formalin (except as noted):

Coagulating gland	Prostate
Kidneys (2)	Seminal vesicles (2)
Liver	Testes with epididymides (2)[a]
Mammary gland (females only)	and vas deferens
Ovaries and oviduct (2)	Uterus[b] with cervix and vagina
Pituitary	All gross lesions[c]

a - Testes and epididymides will be fixed in Bouin's solution.
b - Any uterus stained in 10% ammonium solution for detection of implantation sites will be discarded and will not be preserved in 10% neutral buffered formalin.
c - Representative sections of corresponding organs from a sufficient number of controls will be retained for comparison, if possible.

8.2 Organ Weights:

The following organs will be weighed from all F_0 parental animals euthanized at scheduled termination. Organ-to-final-body weight and organ-to-brain weight ratios will be evaluated.

Brain	Ovaries (with oviducts)
Epididymides*	Pituitary
Kidneys	Testes*
Liver	

* - These paired organs will be weighed separately.

8.3 Microscopic Examination:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the following tissues from all F_0 parental animals from the control and high-dose groups and from all parental animals dying spontaneously or euthanized *in extremis*. If a target organ is identified in the high-dose group, this organ will be examined from all animals in the low and mid-dose groups (at additional cost):

Cervix	Seminal vesicles
Coagulating gland	Testes
Epididymides	Uterus
Ovaries and oviduct	Vagina
Prostate	All gross (internal) lesions

The slides will be prepared by WIL Research Laboratories, LLC and then shipped to Sponsor at the address and contact below for examination by the Principal Investigator, Pathology.

Carolyn Lloyd
DuPont Haskell Global Centers for Health & Environmental Sciences
Investigative Sciences, S320/531
1090 Elkton Road
Newark, DE 19714-0050
Tel: 302-366-5401
Fax: 302-451-4530
Email: carolyn.w.lloyd@usa.dupont.com

The examination of the slides will be performed by the Principal Investigator for Pathology. A final pathology report will be prepared and submitted to WIL Research for inclusion as an appendix in the main study final report. A Quality Assurance and GLP compliance statement signed by the performing laboratory

will be provided to the WIL Study Director for inclusion in the Final Report. The Sponsor is responsible for archiving of raw data associated with the conduct of the pathological examination.

9 DURATION OF STUDY:

The two generations to be studied (parental animals and first generation offspring) will be termed F_0 and F_1, respectively. The conduct of this study will require approximately 22 weeks for acclimation, mating, gestation and lactation of the F_0 generation.

10 STATISTICAL METHODS:

All analyses will be two-tailed for significance levels of 5% and 1%. All means will be presented with standard deviations. All statistical tests will be performed by a computer with appropriate programming as referenced below. The litter, rather than the pup, will be considered as the experimental unit.

10.1 Parental In-Life Data:

Continuous data variables [mean body weights, body weight gains and food consumption at each interval], pre-coital intervals, gestation length, former implantation sites, unaccounted-for sites, mean days of attainment of developmental landmarks (balanopreputial separation and vaginal patency) and the body weight on the day of attainment will be subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor, 1980) to determine intergroup difference. If the results of the ANOVA are significant ($p<0.05$), Dunnett's test (Dunnett, 1964) will be applied to the data to compare the treated groups to the control group.

Male and female mating, fertility, copulation and conception indices of the treated groups will be compared to the control group using the Chi-square test with Yates' correction factor (Hollander, 1999).

10.2 Litter Data:

The mean litter proportions (% per litter) of pup viability during the postnatal period and sex ratio at birth will be subjected to the Kruskal-Wallis nonparametric ANOVA test (Kruskal, 1952) to determine intergroup difference. If the results of the ANOVA are significant ($p<0.05$), the Dunn's Test (Dunn, 1964) will be applied to compare the treated groups to the control group. Mean numbers of pups born, live litter size and litter weights will be subjected to the parametric ANOVA test (Snedecor, 1980) and Dunnett's test (Dunnett, 1964) as described above with the litter representing the experimental unit.

10.3 Histopathology and Organ Weight Data:

Histopathological findings of each treated group will be compared to those of the control group by the Fisher's Exact test (Steel, 1980). Organ weights (absolute and relative to body weights and relative to brain weights) will be subjected to a parametric ANOVA test (Snedecor, 1980) and Dunnett's test (1964) as described above.

11 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with the study protocol and protocol amendments, WIL Standard Operating Procedures and the appropriate provisions of EPA/TSCA and FIFRA Good Laboratory Practice Standards published in the Federal Register (40 CFR Part 792 and 40 CFR Part 160) and the OECD Principles of Good Laboratory Practice. The final report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor Representative to assure that the final report accurately describes the conduct and the findings of the study.

The pathological examination of the slides will be conducted following the Standard Operating Procedures of the performing laboratory and in accordance with GLPs. Quality Assurance monitoring of these analyses for SOP and GLP compliance is the responsibility of the performing laboratory. Inspection reports will be supplied to the Study Director. Upon completion of the prescribed activities and submission of the results to the Sponsor and Study Director the performing laboratory will provide a signed Quality Assurance Statement to the Sponsor (copy to the Study Director). The results will be included in the final report.

This study will be included on the WIL master list of regulated studies.

12 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored as described in Section 13 in the Archives at WIL Research Laboratories, LLC.

The Sponsor will be responsible for the archival of the raw data and records for the pathological examination.

13 WORK PRODUCT:

The Sponsor will have title to all documentation records, raw data, slides, specimens and other work product generated during the performance of the study. Any remaining formulation samples will be discarded after the issuance of the Final Report. All work product, including raw paper data, pertinent electronic storage

media and specimens, will be retained for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

All work product generated at a performing laboratory will be retained at an appropriate archive facility as designated by the SOPs of the performing laboratory.

14 REPORTS:

The final report will contain a summary, test item data, methods and procedures, maternal and pup data WIL Historical Control Data, the analytical chemistry report, pathology report and an interpretation and discussion of the study results. The final report will be comprehensive and shall define level(s) inducing toxic effects as well as no-effect level(s) under the conditions of this investigation. The report will contain all information necessary to conform with current OPPTS and OECD specifications.

WIL Research Laboratories, LLC will submit one copy of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. One revision will be permitted as part of the cost of the study, from which the Sponsor's reasonable revisions and suggestions will be incorporated into the final report, as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL Research Laboratories, LLC within a two-month time frame following submission. WIL Research Laboratories, LLC will submit the final report within one month following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL Research Laboratories, LLC within one year following submission of the draft report, WIL Research Laboratories, LLC may elect to finalize the report following appropriate written notification to the Sponsor. Two electronic copies (PDF) of the final report on CD-R will be provided. Requests for paper copies of the final report may result in additional charges.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act (AWA) regulations (9 CFR Parts 1, 2 and 3). The Sponsor should make particular note of the following:

- The Sponsor Representative's signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.

- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.

- Animals that experience severe pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

17 REFERENCES:

Adams, J.; Buelke-Sam, J.; Kimmel, C.A.; Nelson, C.J.; Reiter, L.W.; Sobotka, T.J.; Tilson, H.A.; Nelson, B.K. Collaborative behavioral teratology study: protocol design and testing procedure. *Neurobehavioral Toxicology and Teratology* **1985**, *7*, 579-586.

Dawson, A.B. A note on the staining of the skeleton of cleared specimens with Alizarin Red S. *Stain Technology* 1926, *1*, 123-124.

Dunn, O.J. Multiple comparisons using rank sums. *Technometrics* 1964, *6(3)*, 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* 1964, *20*, 482-491

Haas, M. A 90-Day Oral (Gavage) Study of H-28548 in Rats with a 28-Day Recovery. WIL-189216, Draft.

Hollander, M.; Wolfe, D.A. *Nonparametric Statistical Methods*, 2nd ed.; Hollander, M., Wolfe, D.A., Eds.; John Wiley and Sons, Inc.: New York, NY, 1999; p 468.

Korenbrot, C.C.; Huhtaniemi, I.T.; Weiner, R.W. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction* 1977, *17*, 298-303.

Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* 1952, *47*, 583-621.

Salewski, E. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. [Staining method for a macroscopic test for implantation sites in the uterus of the rat]. *Naunyn - Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie* 1964, *247*, 367.

Snedecor, G.W.; Cochran, W.G. One Way Classifications; Analysis of Variance. In *Statistical Methods*, 7th ed.; The Iowa State University Press: Ames, IA, 1980; pp 215-237.

Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics, A Biometrical Approach*, 2nd ed.; McGraw-Hill Book Company: New York, NY, 1980; pp 504-506.

Stuckhardt, J.L.; Poppe, S.M. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratogenesis, Carcinogenesis and Mutagenesis* **1984,** *4,* 181-188.

18 PROTOCOL APPROVAL:

Sponsor approval received via email on 4 Jan 2010.
Date

E. I. du Pont de Nemours and Company

Susan M. Munley
Susan M. Munley, MA
Sponsor Representative

8 Jan 2010
Date

WIL Research Laboratories, LLC

Tammye L. Edwards
Tammye L. Edwards, BS, LAT
Study Director

4 Jan 2010
Date

Donald G. Stump
Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

4 Jan 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 1

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) Applicable Protocol Sections: 3

   The proposed audited draft date is 10 September 2010.

2) Applicable Protocol Sections: 7.2

   The control group and three test item groups will consist of 25 males and 25 females each.

3) Applicable Protocol Sections: 7.3

   Appropriately sized flexible, Teflon®-shafted, stainless steel dosing cannulae will be used for the oral administration by gavage. The dosing cannulae may or may not be ball-tipped as appropriate for the age of the animal.

4) Applicable Protocol Sections: 7.4.3.2

   F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 20, inclusively, for females that deliver.

5) Applicable Protocol Sections: 7.6

   Detection of mating will be confirmed by the appearance of a vaginal copulatory plug.

6) Applicable Protocol Sections: 7.9.2.2

For those females with evidence of mating, body weights will be recorded individually on a weekly basis, beginning on the first day of dose administration, until evidence of copulation is observed and on gestation days 0, 4, 7, 11, 14 and 18 and on lactation days 1, 4, 7, 14 and 21.

7) Applicable Protocol Sections: 7.11.1.2

On post-mating day 23 (females with evidence of mating) or post-cohabitation day 23 (females without evidence of copulation), the F_0 females which fail to deliver will be euthanized by carbon dioxide inhalation.

8) Applicable Protocol Sections: 7.12.1

The second sentence of the first paragraph is changed to the following:
A detailed physical examination will be conducted weekly.

9) Applicable Protocol Sections: 7.12.2

F_1 males and females will be have a body weight recorded approximately weekly, beginning with the start of test substance administration until euthanasia (PND 21, 28, 35 and 40).

10) Applicable Protocol Sections: 8.1

Footnote "a" should read:

Testes and epididymides will be fixed in Bouin's solution. Care will be taken to ensure separation between the left and right organs.

11) Applicable Protocol Sections: 8.3

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the listed tissues from all F_0 parental animals from the control and high-dose groups and from all parental animals dying spontaneously or euthanized *in extremis* and from any animals in the low and mid dose groups with impaired fertility (males that did not sire a litter or females that did not deliver a litter).

Reasons for Protocol Modification:

1) Audited report date added to protocol.

2) The number of animals in each dose group was increased to 25 per sex to ensure an adequate number of pregnant females per group.

3) Ball-tipped steel gavage needles are not used on pups under 28 days of age.

4) Clarification of dosing regimen for the females that deliver.

5) Vaginal lavages are not used for the determination of pregnancy in mice, just the presence of copulatory plugs.

6) Correction of gestation days body weights are collected and mice deliver on GD 18.

7) Change in the post-mating or post-cohabitation day that the mice will be euthanized on due to the mouse having a shorter gestation length.

8) F_1 clinical observations were changed to weekly physical examinations for consistency with the F_0 observations.

9) Correction of typographical error.

10) Clarification of maintenance of left and right organ separately for necropsy tissue collection.

11) Addition of microscopic evaluation of animals in the low and mid dose group that have impaired fertility.

Approval:

Sponsor's approval was obtained via email on January 19, 2010.

Tammye L. Edwards, BS, LAT
Study Director

22 Jan 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

22 Jan 2010
Date

Susan M. Munley, MA
Sponsor Representative

29 Jan 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 2

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **5.6 Approximate Age:**

   The approximate age of the males at randomization will be 42-63 days. The approximate age of the females at randomization will be 70-80 days.

2) **6.1 Animal Housing:**

   The females will be housed individually in solid bottom cages upon arrival.

Reasons for Protocol Modification:

1) Age of mice changed to ensure sexual maturity at the time of breeding.

2) The caging upon arrival was changed to individual due to the increase in age of the animal upon arrival.

Approval:

Sponsor's approval was obtained via email on February 10, 2010.

WIL Research Laboratories, LLC

Tammye L. Edwards, BS, LAT
Study Director

12 Feb 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

12 Feb 2010
Date

E.I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

15 Feb 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 3

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **7.9.3.2 Females:**

The first paragraph is changed to the following:

Recorded individually on a weekly basis beginning on the first day of dose administration, until the start of the mating period. Individual food consumption will be recorded on the day evidence of copulation is observed (GD 0) and on gestation days 4, 7, 11, 14 and 18 and lactation days 1, 4, 7, 14 and 21.

Reasons for Protocol Modification:

1) Gestation food consumption intervals corrected for the mouse gestational period.

Approval:

Sponsor's approval was obtained via email on March 11, 2010.

WIL Research Laboratories, LLC

Tammye L. Edwards, BS, LAT
Study Director — 11 March 2010 Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology — 16 Mar 2010 Date

E.I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative — 12 March 2010 Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 4

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **1 Objective:**

   The following is added to this section:

   In addition, a toxicokinetic assessment of plasma levels of the test article will be performed in the F_0 females and the F_1 pups at culling and on PND 21 and PND 40.

2) **2.4 WIL Departmental Responsibilities:**

   The following person is added to this section:

   Carol S. Wally, BA, SRS, RLATG
   Group Supervisor, Sample Processing Laboratory

3) The following sections are added to the protocol:

   2.5 Principal Investigator, Plasma Sample Analysis and Report:

   Michael Mawn, PhD
   Senior Research Chemist
   DuPont Stine-Haskell Research Center
   1090 Elkton Road
   Bldg. S-315 Lab 1333
   Newark, DE 19714-0030
   Tel: 302-451-3365
   Email: michael.p.mawn@usa.dupont.com

4) The following sections are added to the protocol:

7.15 Plasma Sample Collection and Analysis:

7.15.1 Interval:

Blood samples will be collected at the time of scheduled necropsy on LD 21 from 5 randomly selected F_0 females per group that delivered. A blood sample will be collected from all females that failed to deliver on post-mating day 23 at the time of the scheduled necropsy.

In addition, all control females that delivered but were not selected for blood collection as indicated above, will have blood samples taken on LD 21 at the time of scheduled necropsy to provide control animal plasma for method development work to be conducted by the Sponsor. These control samples will be processed and shipped as described for the study samples.

Blood samples will also be collected from the F_1 culled pups on PND 4 from 10 randomly chosen litters in each group following culling and data collection.

On PND 21, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy that are not selected for the F_1 generation.

On PND 40, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy.

7.15.2 Route of Collection:

Blood samples will be collected via the vena cava following euthanasia by carbon dioxide inhalation from the F_0 females and the F_1 PND 21 and PND 40 animals.

Blood samples will be collected via decapitation from the PND 4 pups and pooled by litter.

7.15.3 Target Blood Volume:

For the F_0 females and the F_1 PND 21 and PND 40 animals, 1.0 mL or as much as possible, will be collected into pre-chilled, uniquely-labeled tubes. For the PND 4 pups, blood will be pooled by litter from all the culled pups in each litter to obtain as much blood as possible.

7.15.4 Anticoagulant:

K_3EDTA

7.15.5 Sample Handling and Plasma Preparation:

Samples will be kept on wet ice, protected from light, until centrifugation. All samples will be centrifuged [approximately 3000 rpm (approximately 2060 x g) for approximately 10 min] at approximately 4°C. Plasma will be transferred into new, uniquely-labeled polypropylene tubes.

7.15.6 Label Information:

Samples will include study number, dose group, animal number, interval, sample type and date and time of blood collection.

7.15.7 Storage:

Plasma samples will be stored frozen at approximately -20°C until analysis. The time and date the samples were placed in the freezer will be recorded.

7.15.8 Sample Shipment:

Frozen samples in dry ice, an inventory list and documentation of actual blood collection times for each animal will be shipped on the first Monday or Tuesday after the last sample is collected. The recipient will be notified at least 24 hours in advance of any shipment. Samples will be shipped overnight to:

Michael Mawn, PhD
Senior Research Chemist
DuPont Stine-Haskell Research Center
1090 Elkton Road
Bldg. S-315 Lab 1334
Newark, DE 19714-0030
Tel: 302-451-3365
Email: michael.p.mawn@usa.dupont.com

7.15.9 Plasma Analyses and Report:

Plasma samples will be analyzed for the test article content after solvent protein precipitation with LC/MS/MS analysis. The method of analysis will be documented in the study records and final report. The Principal Investigator for the plasma analysis will be responsible for all bioanalytical delegated-phase activities and will issue a formal bioanalytical/plasma analyses report from the data generated that will be included as an appendix in the final report. A Quality Assurance and GLP compliance statement signed by Sponsor and archival location of the data will be provided to the WIL Study Director for inclusion in the Final Report.

5) **11 Quality Assurance:**

The first sentence of the second paragraph is changed to the following:

The plasma samples analysis and the pathological examination of the slides will be conducted following the Standard Operating Procedures of the performing laboratory and in accordance with GLPs.

6) **12 Records To Be Maintained:**

The second paragraph is changed to the following:

The Sponsor will be responsible for the archival of the raw data and records for the plasma sample analyses and the pathological examination.

7) **13 Work Product:**

The second sentence of the first paragraph is changed to the following:

Any remaining plasma samples and formulation samples will be discarded after the issuance of the Final Report.

8) **14 Reports:**

The second sentence of the first paragraph is changed to the following:

The final report will contain a summary, test item data, methods and procedures, maternal and pup data WIL Historical Control Data, the analytical chemistry report, the plasma analysis report, the pathology report and an interpretation and discussion of the study results.

Reasons for Protocol Modification:

1-8) Blood collection for plasma sample analyses is added to the protocol at the Sponsor's request to characterize the exposure levels of the test substance.

Approval:

Sponsor's approval was obtained via email on April 14, 2010.

WIL Research Laboratories, LLC

Tammye L. Edwards, BS, LAT
Study Director

15 April 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

15 Apr 2010
Date

E. I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

19 Apr 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 5

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **7.4.3.2 Females:**

The first sentence of this section is changed to the following:

F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 20, inclusively, for females that deliver, with the exception of the 5 females/group that are selected for blood collection on LD 21, which will also receive a dose on LD 21.

2) **7.4.3.3 F_1 Males and Females:**

F_1 males and females will be dosed beginning in PND 21 through PND 40, inclusively.

3) **7.15.1 Interval:**

This section is changed to the following:

Blood samples will be collected at 2 hours post dose administration on LD 21 at necropsy from 5 randomly selected F_0 females per group that delivered. A blood sample will be collected from all females that failed to deliver on post-mating day 23 at the time of the scheduled necropsy (not timed).

In addition, all control females that delivered but were not selected for blood collection as indicated above, will have blood samples taken on LD 21 at the time of scheduled necropsy (not timed) to provide control animal plasma for method

development work to be conducted by the Sponsor. These control samples will be processed and shipped as described for the study samples.

Blood samples will also be collected from the F_1 culled pups on PND 4 from 10 randomly chosen litters in each group following culling and data collection.

On PND 21, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy (not timed) that are not selected for the F_1 generation.

On PND 40, blood samples will be collected at 2 hours dose administration at necropsy from 5 randomly selected F_1 males and females in each group.

Reasons for Protocol Modification:

1-3)Per the Sponsor, the most appropriate time of blood collection is 2 hours following dose administration; therefore, an additional dose day for the LD 21 females selected for blood collection and an additional dose day for all F_1 pups on PND 40 was added and the time and days of sample collection was added as appropriate.

Approval:

Sponsor's approval was obtained via email on May 3, 2010.

WIL Research Laboratories, LLC

Tammy L. Edwards, BS, LAT
Study Director

4 May 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental
and Reproductive Toxicology

4 May 2010
Date

E. I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

5 May 2010
Date

PROTOCOL

AN ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY OF H-28548 IN MICE

(U.S. EPA OPPTS 870.3550 and OECD Guideline 421)

Submitted To:

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898

DuPont Work Request Number: 18405
DuPont Service Code: 1037
DuPont Study Number: 18405-1037

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

ED_002003A_00062143-00001

1 OBJECTIVE:

To provide preliminary information on the potential adverse effects of the test substance on male and female reproduction within the scope of a screening study. This will encompass gonadal function, mating behavior, conception, parturition and lactation of the F_0 generation and the development of offspring from conception through day 40 of postnatal life.

In addition, a toxicokinetic assessment of plasma levels of the test article will be performed in the F_0 females and the F_1 pups at culling and on PND 21 and PND 40.

This study is subject to the applicable regulations of the Organisation for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Guideline 421, Reproduction/Development Toxicity Screening Test, July 27, 1995, and the United States Environmental Protection Agency (EPA) Health Effects Test Guidelines OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test, July 2000 and will be conducted in accordance with the EPA/TSCA and FIFRA (40 CFR Part 792 and 40 CFR Part 160) and the OECD Principles of Good Laboratory Practice.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Study Representative:

Susan M. Munley, MA
Research Toxicologist
Developmental, Reproductive and Neurobehavioral Toxicology
DuPont Haskell Laboratory for Health and Environmental Sciences
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2.4 WIL Departmental Responsibilities:

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Carol S. Wally, BA, SRS, RLATG
Group Supervisor, Sample Processing Laboratory

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2.5 Principal Investigator, Plasma Sample Analysis and Report:

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3 STUDY SCHEDULE:

Proposed Experimental Starting (Animal Receipt) Date:	5 January 2010
Proposed Experimental Start (First Day of Dosing) Date:	14 January 2010
Proposed Experimental Completion/Termination Date:	4 June 2010
Proposed Audited Report Date:	~~To be determined~~10 September 2010

4 TEST SUBSTANCE DATA:

4.1 Test Substance Shipment:

Test substance and applicable documentation, including a Certificate of Analysis, will be shipped under Sponsor's responsibility to:

Formulations Laboratory (WIL-189225; Tammye Edwards)
Attn: Larry Blessing
WIL Research Laboratories, LLC
1407 George Road
Ashland, Ohio 44805-8946

4.2 Identification:

H-28548 or HFPO Dimer Acid Ammonium Salt

4.3 Haskell Test Substance Number:

H-28548

4.4 Lot Number:

E109540-44A

4.5 Expiration/Retest Date:

13 June 2011

4.6 Purity:

84%

4.7 Storage Conditions:

Controlled room temperature and humidity (approximately 18° to 24°C and 20% to 70% relative humidity)

4.8 Stability:

The analysis was performed by the Sponsor and documented on the Certificate of Analysis.

4.9 Physical Description:

To be documented by WIL Research Laboratories, LLC.

4.10 Reserve Samples:

Reserve samples of the test substance will be taken in accordance with WIL Standard Operating Procedures and stored in the Archives at WIL Research Laboratories, LLC indefinitely, unless otherwise specified.

4.11 Personnel Safety Data:

See the Material Safety Data Sheet (MSDS) provided by the Sponsor.

4.12 Test Substance Disposition:

With the exception of the reserve sample for each batch of test substance, which will be archived as described, all neat test substance remaining at completion of the in-life phase of the study will be kept for subsequent studies.

5 TEST SYSTEM:

5.1 Species:

Mouse

5.2 Strain:

Charles River Crl:CD1(ICR)

5.3 Source:

Males: Charles River Laboratories, Inc., Raleigh, NC
Females: Charles River Laboratories, Inc., Kingston, NY

5.4 Number on Study:

100 males and 100 females (minimum of 120 males and 120 females purchased; males and females will be ordered from separate facilities to ensure the avoidance of sibling mating). Animals not assigned to study will be transferred to the stock animal colony or will be euthanized by carbon dioxide inhalation and the carcasses discarded.

The number of animals used on this study is consistent with OPPTS and OECD guidelines for reproduction/developmental toxicity screening studies.

5.5 Body Weight Range:

A minimum of 20 grams at randomization.

5.6 Approximate Age:

The approximate age of the males at randomization will be 42-63 days. The approximate age of the females at randomization will be 70-80 days.~~42-63 days old at randomization.~~

5.7 Identification System:

Each mouse will be uniquely identified by tattoo markings applied to the tail. Individual cage cards will be affixed to each cage and will display the animal number, group number, study number, dosage level and sex of the animal.

5.8 Justification for Selection:

This species and strain of animal is recognized as appropriate for reproduction studies. WIL Research Laboratories, LLC has reproductive historical control data in the Crl:CD1(ICR) mouse. This animal model has been proven to be susceptible to the effects of reproductive toxicants.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed, 2-3 per cage, for at least 3 days following receipt. Thereafter, the mice will be housed individually. The females will be housed individually in solid bottom cages upon arrival.The F_0 males and females will be individually housed in solid bottom cages (plastic maternity cages) containing ground corncob nesting material (Bed-O' Cobs®) in an environmentally controlled room during the quarantine period and throughout the entire study until euthanasia. All F_1 offspring not euthanized at weaning will be housed by litter in the plastic cages with nesting material until postnatal day (PND) 28. F_1 offspring not selected for the maturation phase will be necropsied on PND 21. On PND 28, F_1 offspring will be individually housed in solid bottom cages (plastic maternity cages) containing ground corncob nesting material (Bed-O' Cobs®). The cages will be subject to routine cleaning at a frequency consistent with maintaining good animal health and WIL Standard Operating Procedures. The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain temperature at 71 ± 5°F (22 3°C) and relative humidity at 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic

collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Reverse osmosis-purified water will be available *ad libitum*. Filters servicing the automatic watering system are changed regularly according to WIL Standard Operating Procedures. The municipal water supplying the laboratory is analyzed according to WIL Standard Operating Procedures on a routine basis to ensure that contaminants are not present in concentrations that would be expected to affect the outcome of the study.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 will be offered *ad libitum* during the study. Periodic analyses of the certified feed are performed by the manufacturer to ensure that heavy metals and pesticides are not present at concentrations that would be expected to affect the outcome of the study. Results of the analyses are provided to WIL Research Laboratories, LLC by the manufacturer. Feeders will be changed and sanitized once per week.

6.5 Enrichment:

All animals will be offered Nestlets™ for enrichment that will be replaced as needed.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Quarantine:

Each animal will be inspected by a qualified technician upon receipt. Mice judged to be in good health and suitable as test animals will be immediately placed in quarantine for a minimum of 9 days. All mice will be initially weighed, permanently identified by tattoo markings applied to the tail and receive a clinical observation. During the quarantine period, each mouse will be observed twice daily for changes in general appearance and behavior. Prior to the start of the in-life phase, those animals judged to be suitable test subjects will be identified and receive a detailed physical examination.

7.2 Randomization:

At the conclusion of the quarantine period, animals judged to be suitable test subjects and meeting acceptable body weight requirements, will be assigned at

random using a computer program. At that time, the animal numbers and corresponding body weights will be entered into the WIL Toxicology Data Management System (WTDMS). A printout containing the animal numbers and individual group assignments will be generated based on body weight stratification into a block design. Animals will then be arranged into the groups according to the printout. The control group and three test item groups will consist of ~~20~~ 25 males and ~~20~~ 25 females each.

Any animal assigned to the study that is found dead, euthanized *in extremis* or exhibits abnormal clinical signs, reduced food consumption or body weight losses prior to the start of dosing may be replaced by an animal of appropriate age when possible. Replacement animals will be arbitrarily assigned (not computer randomized) to the study based on comparable body weights (if possible) with respect to the animal that was replaced.

7.3 Route and Rationale of Test Item Administration:

The route of administration will be oral (gavage). Historically, this route has been used extensively for studies of this nature. Appropriately sized flexible, Teflon®-shafted, stainless steel dosing cannulae will be used for the oral administration by gavage. The dosing cannulae mayor may not be ball-tipped as appropriate for the age of the animal.~~Appropriately sized flexible, Teflon® shafted, stainless steel balltipped dosing cannulae will be used for the oral administration by gavage~~.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The dose levels proposed for the current study are 0, 0.1, 0.5, and 5 mg/kg/day and are based on previous and ongoing general toxicity studies in mice. These levels are currently being tested in an ongoing (in-life dosing phase complete) subchronic toxicity 90-day gavage study (DuPont-18405-1307). The doses for the 90-day gavage study were based on results from a previous 28-day gavage study (DuPont-24459) in which doses of 0, 0.1, 3, and 30 mg/kg/day were tested.

The following table presents the study group arrangement.

Group Number	Test Item	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Male	Female
1	Vehicle Control[b]	0	0	10	25	25
2	H-28548	0.1	0.01	10	25	25
3	H-28548	0.5	0.05	10	25	25

4	H-28548	5	0.5	10	25	25

[a] Dosage levels will be corrected for the purity of 84%.
[b] Deionized Water

7.4.2 Vehicle Control Item:

Deionized Water

7.4.3 F_0 Treatment Regimen:

The test and control items will be administered once daily at approximately the same time each day as follows:

7.4.3.1 Males:

F_0 males will be dosed for a minimum of 70 days prior to mating and continuing until the day prior to the scheduled euthanasia.

7.4.3.2 Females:

F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 20, inclusively, for females that deliver, with the exception of the 5 females/group that are selected for blood collection on LD 21, which will also receive a dose on LD 21. ~~F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 21 for females that deliver. For females that do not have positive signs of mating or delivery, dosing will continue until one day prior to euthanasia~~.

7.4.3.3 F_1 Males and Females:

F_1 males and females will be dosed beginning in PND 21 through PND 40, inclusively. ~~F_1 males and females will be dosed beginning in PND 21 until one day prior to euthanasia.~~

7.4.4 Adjustment of Dosages:

Individual dosages will be calculated based on the most recent body weight to provide the proper mg/kg/day dosage.

7.5 Preparation and Analysis of Test Item Formulations:

7.5.1 Method and Frequency of Preparation:

Based on the physical characteristics of the test substance, appropriate methods will be used to ensure the best possible formulations of the test substance in the vehicle. Dosing formulations will be stored refrigerated (2-8°C) for a maximum of 12 days. The Study Director or designee will visually inspect the formulations prior to the initiation of dosing. This visual inspection will be performed to ensure that the formulations are visibly homogeneous and acceptable for dosing. Any special procedures required for formulation will be documented according to Good Laboratory Practices and presented in the final report of this study. Test substance formulations will be prepared approximately weekly and divided into aliquots for daily dispensation. The test substance and vehicle formulations will be stirred continuously during dosing.

7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Substance Formulations:

Stability and resuspension homogeneity were established on a previous study (Haas, Draft; WIL-189216). Test substance formulations were stable and 12 days of room temperature storage or refrigerated storage (2-8°C) at concentrations of 0.01 mg/mL and 100 mg/mL and homogenous following resuspension after 12 days of refrigerated storage (2-8°C). Stability and resuspension homogeneity will not be conducted on this study.

Homogeneity and concentration will be conducted on the first formulations prepared for dosing. Four 1-mL samples will be collected from the top, middle and bottom of the test substance formulations from the low and high dose groups and the samples analyzed to assess the homogeneity of the test substance in the mixtures; the middle strata will serve as the measure of test substance concentration. Four 1-mL samples will be taken from the middle on the control and the mid-dose groups and analyzed for concentration of the test substance.

Concentration will be assessed on Week 4, 8, 12, 16 and 19 formulations prepared for dosing. Four 1-mL samples will be collected from the middle of each test substance formulation and the control group and analyzed for test substance content.

7.5.3 Sample Analysis:

Samples will be transferred to the Analytical Chemistry Department at WIL Research Laboratories, LLC for analysis. Analyses of test article formulations will be performed using a method developed and validated

by WIL Research Laboratories, LLC. Initially, two of each set of four replicate, 1-mL samples will be analyzed; the remaining two 1-mL samples will be stored frozen (approximately -20°C) at WIL and will function as back-up samples. Back-up samples will be analyzed if requested by the Sponsor or Study Director or may be discarded following results that are within specifications and approval of the Study Director.

7.6 F_0 Breeding:

After a minimum of 70 days for males and 14 days of exposure for females, of exposure, one female will be cohabitated with one male mouse of the same treatment group, avoiding sibling mating, in a plastic cage for mating. Detection of mating will be confirmed by ~~evidence of sperm in the vaginal lavage~~the appearance of a vaginal copulatory plug. After confirmation of mating, the female will be returned to an individual plastic cage and the day will be designated as day 0 of gestation.

A maximum of 14 days will be allowed for mating. After 14 days of mating, any females who have not shown evidence of breeding will be placed in a plastic cage containing nesting material.

7.7 F_0 Parturition and Lactation and F_1 Litters:

The day parturition is initiated will be designated as day 0 of lactation. Any difficulties at the time of parturition will be recorded. When parturition is judged to be complete, the sex of each pup will be determined, pups will be examined for gross malformations and the number of stillbirths and live pups will be recorded. Any changes or abnormalities in nesting and nursing behavior will be recorded. The dam and litter will remain together until postnatal day (PND) 21.

7.8 Identification of F_1 Litters:

Upon completion of delivery, all pups will be individually identified by tattoo markings applied to the digits. To reduce variability among the litters, on PND 4, eight pups of equal sex distribution (if possible) from each litter will be randomly selected. For litters consisting of fewer than eight pups, adjustments for litter sizes will not be performed. Following selection, the non-selected PND 4 pups will be euthanized by an intraperitoneal injection of sodium pentobarbital and discarded.

7.9 General Observations During the Experimental Period

7.9.1 Parental Appearance and Behavior:

Each parental mouse (F_0) will be observed twice daily for moribundity and mortality, once in the morning and once in the afternoon. A detailed physical examination will be conducted weekly. Mortality and all signs of overt toxicity will be recorded on the day observed. The observations shall include, but are not limited to, evaluations for changes in appearance of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior. During the period of expected parturition, the dams will be observed twice daily for dystocia, prolonged labor, delayed labor or other difficulties at parturition. All animals will also be observed on the day of necropsy and findings will be recorded.

During the treatment period, each animal will be observed at approximately 1-2 hours following each dose administration for findings that are potentially related to treatment of that might change before the next scheduled observation. Additional post dosing observation periods may be necessary and will be documented in the study records.

7.9.2 Parental Body Weights:

All animals will have a final body weight recorded on the day of euthanasia.

7.9.2.1 Males:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until euthanasia.

7.9.2.2 Females:

For those females with evidence of mating, body weights will be recorded individually on a weekly basis, beginning on the first day of dose administration, until evidence of copulation is observed and on gestation days 0, 4, 7, 11, 14 and 18 and on lactation days 1, 4, 7, 14 and 21~~Recorded individually on a weekly basis, beginning on the first day of dose administration, until evidence of copulation is observed and on gestation days 0, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.~~

For females with no evidence of mating, individual body weights will continue to be recorded on a weekly basis until euthanasia.

7.9.3 Parental Food Consumption*:

Individual food consumption will not be recorded during the breeding period because the animals are cohabitated at that time.

7.9.3.1 Males:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until euthanasia.

7.9.3.2 Females:

Recorded individually on a weekly basis beginning on the first day of dose administration, until the start of the mating period. Individual food consumption will be recorded on the day evidence of copulation is observed (GD 0) and on gestation days 4, 7, 11, 14 and 18 and lactation days 1, 4, 7, 14 and 21. ~~Recorded individually on a weekly basis beginning on the first day of dose administration, until the start of the mating period. Individual food consumption will be recorded on the day evidence of copulation is observed (GD 0) and on gestation days 1, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.~~

For females with no evidence of mating, individual food consumption will continue to be recorded on a weekly basis following the end of the mating period until euthanasia.

7.9.4 Examination of Offspring:

7.9.4.1 Appearance and Behavior:

All pups will be observed daily for general appearance and behavior and survival during lactation. A detailed physical examination will be recorded for each pup on PND 1,4, 7, 14 and 21. Any abnormalities in nesting and nursing behavior will be recorded. The pups will be sexed on PND 0, 4, 14 and 21.

7.9.4.2 Body Weights:

Each pup will be weighed on PND 1, 4, 7, 14 and 21.

7.9.5 Pup Deaths:

7.9.5.1 Pups 0 to 4 Days of Age:

Moribund pups will be euthanized by an intraperitoneal injection of sodium pentobarbital. Stillborn pups, pups found dead between birth and PND 4, and any pups that are euthanized *in extremis* will be dissected (including the heart and the brain examined by a mid-coronal slice) by a technique described by Stuckhardt and Poppe (Stuckhardt and Poppe, 1984). If a skeletal anomaly is suspected, the pups will be eviscerated, cleared and stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined. Representative specimens with malformations may be preserved in 10% neutral buffered formalin at the discretion of the study director.

7.9.5.2 Pups 5 Days of Age to Weaning:

Moribund pups will be euthanized by an intraperitoneal injection of sodium pentobarbital (prior to PND 11) or by carbon dioxide inhalation. A gross necropsy will be performed on pups found dead or euthanized *in extremis*, and gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin. If a skeletal anomaly is suspected, the pups will be eviscerated, cleared and stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined.

7.10 Selection of F_1 Generation and Termination of PND 21 Nonselected Pups:

One male and one female pup per litter will be selected for the F_1 generation on or prior to PND 21. Only pups not expected to survive due to notable physical limitations will not be available for selection. A detailed evaluation of each pup excluded from selection will be recorded.

All PND 21 pups not selected for the F_1 generation will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

7.11 Euthanasia of F_0 Generation:

7.11.1 Females:

7.11.1.1 Females Which Deliver:

On lactation day 21, all F_0 females that delivered will be euthanized by carbon dioxide inhalation. A gross examination will be performed and tissues preserved as described in Section 8.1. The number of former implantation sites will be recorded. Organ weights will be collected and tissues preserved as described in Section 8.2.

7.11.1.2 Females Which Fail to Deliver:

On post-mating day 23 (females with evidence of mating) or post-cohabitation day 23 (females without evidence of copulation), the F_0 females which fail to deliver will be euthanized by carbon dioxide inhalation. ~~On post-mating day 25 (females with evidence of copulation) or post-cohabitation day 25 (females without evidence of copulation), the F_0 females which fail to deliver will be euthanized by carbon dioxide inhalation.~~ A gross necropsy examination will be performed and tissues will be preserved as described in Section 8.1. Organ weights will be collected as described in Section 8.2 with the exception of any ammonium sulfide stained uterus, which will be discarded. Uteri which appear nongravid by macroscopic examination will be opened and placed in a 10% ammonium sulfide solution (Salewski, 1964) for detection of early implantation loss.

7.11.1.3 Females with Total Litter Loss:

Females with total litter loss will be euthanized by carbon dioxide inhalation on the same day. The number of former implantation sites will be recorded and the number of corpora lutea (if litter loss occurs on or before PND 4) will be recorded. A gross necropsy examination will be performed and tissues preserved as described in Section 8.1. Organ weights will be collected as described in Section 8.2.

7.11.1.4 F_0 Deaths and Animals Euthanized *in Extremis*:

Females not surviving until the scheduled euthanasia will have a gross necropsy examination performed and tissues preserved as described in Section 8.1. Animals not expected to survive to the next observation period (moribund) will be euthanized by carbon

dioxide inhalation and have a gross necropsy examination performed and tissues preserved as described in Section 8.1. Organ weights will not be collected from found dead or euthanized *in extremis* females. The number and location of implantation sites or scars will be recorded for females dying or euthanized during gestation and lactation. The number of corpora lutea will be recorded for females dying or euthanized during gestation and up to and including lactation day 4. Uteri which appear nongravid by macroscopic examination will be opened and placed in a 10% ammonium sulfide solution (Salewski, 1964) for detection of early implantation loss.

Viable fetuses will be euthanized by an intrathoracic injection of sodium pentobarbital. Recognizable fetuses will be examined externally for gross abnormalities. Representative specimens with malformations may be preserved in 10% neutral-buffered formalin, at the discretion of the study director. For females found dead or euthanized *in extremis* during lactation, all pups will be examined externally and subjected to a necropsy examination according to Section 7.9.5.

7.11.2 Males:

Following completion of the mating period, all F_0 males will be euthanized by carbon dioxide inhalation and subjected to a gross necropsy and tissue preservation as described in Section 8.1. Organ weights will be collected as described in Section 8.2.

Males not surviving until the scheduled euthanasia will be subjected to a gross necropsy and tissue preservation as described in Section 8.1. Any males not expected to survive to the next observation period (moribund) will be euthanized by carbon dioxide inhalation and also necropsied and have tissues preserved as described in Section 8.1. Organ weights will not be collected.

7.12 F_1 Generation General Observations During The Experimental Period:

7.12.1 F_1 Clinical Observations:

Following weaning and selection, the mice will be observed twice daily for moribundity and mortality, once in the morning and once in the afternoon. ~~Clinical observations will be recorded daily~~A detailed physical examinations will be conducted weekly. Mortality and all signs of overt toxicity will be recorded on the day observed. The observations shall include, but are not limited to, evaluation for changes in

appearance of the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity and behavior patterns. All animals will also be observed on the day of necropsy and any findings will be recorded.

During the treatment period, each animal will be observed at approximately 1-2 hours following each dose administration for findings that are potentially related to treatment of that might change before the next scheduled observation. Additional post dosing observation periods may be necessary and will be documented in the study records.

7.12.2 F_1 Body Weights and Food Consumption:

F_1 males and females will be have a body weight recorded approximately weekly, beginning with the start of test ~~diet~~ substance administration until euthanasia (PND 21, 28, 35 and 40). All animals will have a final body weight recorded on the day of euthanasia.

F_1 males and females will have food consumption recorded individually on an approximately weekly basis beginning on PND 28 until euthanasia (PND 28, 35 and 40). Food consumption will not be collected from PND 21 to PND 28 during group housing for the F_1 males and females.

7.13 F_1 Postweaning Developmental Landmarks:

Offspring selected for the F_1 generation will be evaluated for attainment of the following landmarks of sexual maturity:

7.13.1 Balanopreputial Separation:

Each male pup will be observed for balanopreputial separation beginning on PND 25 as described by Korenbrot *et al.* (Korenbrot 1977). Examination of the males will continue daily until balanopreputial separation is present. The body weight of each male will be recorded on the day of attainment of balanopreputial separation.

7.13.2 Vaginal Patency:

Each female pup will be observed for vaginal patency beginning on PND 21 (only those selected for the F_1 generation) as described by Adams *et al.* (Adams 1985). Examination of the females will continue daily until vaginal patency is present. The body weight of each female will be recorded on the day of attainment of vaginal patency.

7.14 Euthanasia of F_1 Generation:

7.14.1 Scheduled Necropsy

On PND 40, all F_1 animals will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

7.14.2 Unscheduled Deaths or Animals Euthanized *in Extremis*

Any F_1 animals not surviving until the scheduled euthanasia or not expected to survive to the next observation period (euthanized by carbon dioxide inhalation) will be necropsied. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

7.15 Plasma Sample Collection and Analysis:

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7.15.1 Interval:

Blood samples will be collected at 2 hours post dose administration on LD 21 at necropsy from 5 randomly selected F_0 females per group that delivered. A blood sample will be collected from all females that failed to deliver on post-mating day 23 at the time of the scheduled necropsy (not timed).

In addition, all control females that delivered but were not selected for blood collection as indicated above, will have blood samples taken on LD 21 at the time of scheduled necropsy (not timed) to provide control animal plasma for method development work to be conducted by the Sponsor. These control samples will be processed and shipped as described for the study samples.

Blood samples will also be collected from the F_1 culled pups on PND 4 from 10 randomly chosen litters in each group following culling and data collection.

On PND 21, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy (not timed) that are not selected for the $_1$ generation.

On PND 40, blood samples will be collected at 2 hours dose administration at necropsy from 5 randomly selected F_1 males and females in each group.

7.15.2 Route of Collection:

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Blood samples will be collected via the vena cava following euthanasia by carbon dioxide inhalation from the F_0 females and the F_1 PND 21 and PND 40 animals.

Blood samples will be collected via decapitation from the PND 4 pups and pooled by litter.

7.15.3 Target Blood Volume:

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For the F_0 females and the F_1 PND 21 and PND 40 animals, 1.0 mL or as much as possible, will be collected into pre-chilled, uniquely-labeled tubes. For the PND 4 pups, blood will be pooled by litter from all the culled pups in each litter to obtain as much blood as possible.

7.15.4 Anticoagulant:

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K_3EDTA

7.15.5 Sample Handling and Plasma Preparation:

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Samples will be kept on wet ice, protected from light, until centrifugation. All samples will be centrifuged [approximately 3000 rpm (approximately 2060 x g) for approximately 10 min] at approximately 4°C. Plasma will be transferred into new, uniquely-labeled polypropylene tubes.

7.15.6 Label Information:

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Samples will include study number, dose group, animal number, interval, sample type and date and time of blood collection.

7.15.7 Storage:

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Plasma samples will be stored frozen at approximately -20°C until analysis. The time and date the samples were placed in the freezer will be recorded.

7.15.8 Sample Shipment:

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Frozen samples in dry ice, an inventory list and documentation of actual blood collection times for each animal will be shipped on the first Monday or Tuesday after the last sample is collected. The recipient will be notified at least 24 hours in advance of any shipment. Samples will be shipped overnight to:

Michael Mawn, PhD
Senior Research Chemist
DuPont Stine-Haskell Research Center
1090 Elkton Road
Bldg. S-315 Lab 1334
Newark, DE 19714-0030
Tel: 302-451-3365
Email: michael.p.mawn@usa.dupont.com

7.15.9 Plasma Analyses and Report:

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Plasma samples will be analyzed for the test article content after solvent protein precipitation with LC/MS/MS analysis. The method of analysis will be documented in the study records and final report. The Principal Investigator for the plasma analysis will be responsible for all bioanalytical delegated-phase activities and will issue a formal bioanalytical/plasma analyses report from the data generated that will be included as an appendix in the final report. A Quality Assurance and GLP compliance statement signed by Sponsor and archival location of the data will be provided to the WIL Study Director for inclusion in the Final Report.

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8 ANATOMIC PATHOLOGY:

8.1 Macroscopic Examination:

A complete necropsy will be conducted on all F_0 parental animals dying spontaneously, euthanized *in extremis* (by carbon dioxide inhalation) or at termination. This will include examination of the external surface, all orifices, the cranial cavity, the external surface of the brain and the thoracic, abdominal

and pelvic cavities including viscera. For F_0 females, the number of former implantation sites will be recorded.

At the time of necropsy, the following tissues and organs will be collected and placed in 10% neutral-buffered formalin (except as noted):

Coagulating gland	Prostate
Kidneys (2)	Seminal vesicles (2)
Liver	Testes with epididymides (2)[a]
Mammary gland (females only)	and vas deferens
Ovaries and oviduct (2)	Uterus[b] with cervix and vagina
Pituitary	All gross lesions[c]

a - Testes and epididymides will be fixed in Bouin's solution. Care will be taken to ensure separation between the left and right organs

b - Any uterus stained in 10% ammonium solution for detection of implantation sites will be discarded and will not be preserved in 10% neutral buffered formalin.

c - Representative sections of corresponding organs from a sufficient number of controls will be retained for comparison, if possible.

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8.2 Organ Weights:

The following organs will be weighed from all F_0 parental animals euthanized at scheduled termination. Organ-to-final-body weight and organ-to-brain weight ratios will be evaluated.

Brain	Ovaries (with oviducts)
Epididymides*	Pituitary
Kidneys	Testes*
Liver	

* - These paired organs will be weighed separately.

8.3 Microscopic Examination:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the listed tissues from all F_0 parental animals from the control and high-dose groups and from all parental animals dying spontaneously or euthanized *in extremis* and from any animals in the low and mid dose groups with impaired fertility (males that did not sire a litter or females that did not deliver a litter). ~~Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the following tissues from all F_0 parental animals from the control and high-dose groups and from all parental animals dying spontaneously or euthanized *in extremis*.~~ If a target organ is identified in the high-dose group, this organ will be examined from all animals in the low and mid-dose groups (at additional cost):

Cervix	Seminal vesicles

Coagulating gland	Testes
Epididymides	Uterus
Ovaries and oviduct	Vagina
Prostate	All gross (internal) lesions

The slides will be prepared by WIL Research Laboratories, LLC and then shipped to Sponsor at the address and contact below for examination by the Principal Investigator, Pathology.

Carolyn Lloyd
DuPont Haskell Global Centers for Health & Environmental Sciences
Investigative Sciences, S320/531
1090 Elkton Road
Newark, DE 19714-0050
Tel: 302-366-5401
Fax: 302-451-4530
Email: carolyn.w.lloyd@usa.dupont.com

The examination of the slides will be performed bythe Principal Investigator for Pathology. A final pathology report will be prepared and submitted to WIL Research for inclusion as an appendix in the main study final report. A Quality Assurance and GLP compliance statement signed by the performing laboratory will be provided to the WIL Study Director for inclusion in the Final Report. The Sponsor is responsible for archiving of raw data associated with the conduct of the pathological examination.

9 DURATION OF STUDY:

The two generations to be studied (parental animals and first generation offspring) will be termed F_0 and F_1, respectively. The conduct of this study will require approximately 22 weeks for acclimation, mating, gestation and lactation of the F_0 generation.

10 STATISTICAL METHODS:

All analyses will be two-tailed for significance levels of 5% and 1%. All means will be presented with standard deviations. All statistical tests will be performed by a computer with appropriate programming as referenced below. The litter, rather than the pup, will be considered as the experimental unit.

10.1 Parental In-Life Data:

Continuous data variables [mean body weights, body weight gains and food consumption at each interval], pre-coital intervals, gestation length, former implantation sites, unaccounted-for sites, mean days of attainment of

developmental landmarks (balanopreputial separation and vaginal patency) and the body weight on the day of attainment will be subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor, 1980) to determine intergroup difference. If the results of the ANOVA are significant ($p<0.05$), Dunnett's test (Dunnett, 1964) will be applied to the data to compare the treated groups to the control group.

Male and female mating, fertility, copulation and conception indices of the treated groups will be compared to the control group using the Chi-square test with Yates' correction factor (Hollander, 1999).

10.2 Litter Data:

The mean litter proportions (% per litter) of pup viability during the postnatal period and sex ratio at birth will be subjected to the Kruskal-Wallis nonparametric ANOVA test (Kruskal, 1952) to determine intergroup difference. If the results of the ANOVA are significant ($p<0.05$), the Dunn's Test (Dunn, 1964) will be applied to compare the treated groups to the control group. Mean numbers of pups born, live litter size and litter weights will be subjected to the parametric ANOVA test (Snedecor, 1980) and Dunnett's test (Dunnett, 1964) as described above with the litter representing the experimental unit.

10.3 Histopathology and Organ Weight Data:

Histopathological findings of each treated group will be compared to those of the control group by the Fisher's Exact test (Steel, 1980). Organ weights (absolute and relative to body weights and relative to brain weights) will be subjected to a parametric ANOVA test (Snedecor, 1980) and Dunnett's test (1964) as described above.

11 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with the study protocol and protocol amendments, WIL Standard Operating Procedures and the appropriate provisions of EPA/TSCA and FIFRA Good Laboratory Practice Standards published in the Federal Register (40 CFR Part 792 and 40 CFR Part 160) and the OECD Principles of Good Laboratory Practice. The final report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor Representative to assure that the final report accurately describes the conduct and the findings of the study.

The plasma samples analysis and the pathological examination of the slides will be conducted following the Standard Operating Procedures of the performing laboratory and in accordance with GLPs.~~The pathological examination of the slides will be conducted following the Standard Operating Procedures of the performing laboratory and in accordance with GLPs.~~ Quality Assurance monitoring of these analyses for SOP and GLP compliance is the responsibility of the performing laboratory. Inspection reports will be supplied to the Study Director. Upon completion of the prescribed activities and submission of the results to the Sponsor and Study Director the performing laboratory will provide a signed Quality Assurance Statement to the Sponsor (copy to the Study Director). The results will be included in the final report.

This study will be included on the WIL master list of regulated studies.

12 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored as described in Section 13 in the Archives at WIL Research Laboratories, LLC.

The Sponsor will be responsible for the archival of the raw data and records for the plasma sample analyses and the pathological examination.~~The Sponsor will be responsible for the archival of the raw data and records for the pathological examination.~~

13 WORK PRODUCT:

The Sponsor will have title to all documentation records, raw data, slides, specimens and other work product generated during the performance of the study. Any remaining plasma samples and formulation samples will be discarded after the issuance of the Final Report. ~~Any remaining formulation samples will be discarded after the issuance of the Final Report.~~ All work product, including raw paper data, pertinent electronic storage media and specimens, will be retained for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

All work product generated at a performing laboratory will be retained at an appropriate archive facility as designated by the SOPs of the performing laboratory.

14 REPORTS:

The final report will contain a summary, test item data, methods and procedures, maternal and pup data WIL Historical Control Data, the analytical chemistry report, the plasma analysis report, the pathology report and an interpretation and discussion of the study results. ~~The final report will contain a summary, test item data, methods and procedures, maternal and pup data WIL Historical Control Data, the analytical chemistry report, pathology report and an interpretation and discussion of the study results.~~ The final report will be comprehensive and shall define level(s) inducing toxic effects as well as no-effect level(s) under the conditions of this investigation. The report will contain all information necessary to conform with current OPPTS and OECD specifications.

WIL Research Laboratories, LLC will submit one copy of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. One revision will be permitted as part of the cost of the study, from which the Sponsor's reasonable revisions and suggestions will be incorporated into the final report, as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL Research Laboratories, LLC within a two-month time frame following submission. WIL Research Laboratories, LLC will submit the final report within one month following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL Research Laboratories, LLC within one year following submission of the draft report, WIL Research Laboratories, LLC may elect to finalize the report following appropriate written notification to the Sponsor. Two electronic copies (PDF) of the final report on CD-R will be provided. Requests for paper copies of the final report may result in additional charges.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act (AWA) regulations (9 CFR Parts 1, 2 and 3). The Sponsor should make particular note of the following:

The Sponsor Representative's signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.

Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.

Animals that experience severe pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

17 REFERENCES:

Adams, J.; Buelke-Sam, J.; Kimmel, C.A.; Nelson, C.J.; Reiter, L.W.; Sobotka, T.J.; Tilson, H.A.; Nelson, B.K. Collaborative behavioral teratology study: protocol design and testing procedure. *Neurobehavioral Toxicology and Teratology* **1985,** *7,* 579-586.

Dawson, A.B. A note on the staining of the skeleton of cleared specimens with Alizarin Red S. *Stain Technology* **1926,** *1,* 123-124.

Dunn, O.J. Multiple comparisons using rank sums *Technometrics* **1964,** *6(3),* 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* **1964,** *20,* 482-491

Haas, M. A 90-Day Oral (Gavage) Study of H-28548 in Rats with a 28-Day Recovery. WIL-189216, **Draft.**

Hollander, M.; Wolfe, D.A. *Nonparametric Statistical Methods,* 2nd ed.; Hollander, M., Wolfe, D.A., Eds.; John Wiley and Sons, Inc.: New York, NY, **1999;** p 468.

Korenbrot, C.C.; Huhtaniemi, I.T.; Weiner, R.W. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction* **1977,** *17,* 298-303.

Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* **1952,** *47,* 583-621.

Salewski, E. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. [Staining method for a macroscopic test for implantation sites in the uterus of the rat]. *Naunyn - Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie* **1964,** *247,* 367.

Snedecor, G.W.; Cochran, W.G. One Way Classifications; Analysis of Variance. In *Statistical Methods,* 7th ed.; The Iowa State University Press: Ames, IA, **1980;** pp 215-237.

Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics, A Biometrical Approach,* 2nd ed.; McGraw-Hill Book Company: New York, NY, **1980;** pp 504-506.

Stuckhardt, J.L.; Poppe, S.M. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratogenesis, Carcinogenesis and Mutagenesis* **1984,** *4,* 181-188.

18 PROTOCOL APPROVAL:

Sponsor approval received via _____ on _____.
Date

E. I. du Pont de Nemours and Company

_____ _____
Susan M. Munley, MA Date
Sponsor Representative

WIL Research Laboratories, LLC

_____ _____
Tammye L. Edwards, BS, LAT Date
Study Director

_____ _____
Donald G. Stump, PhD, DABT Date
Director, Developmental and
Reproductive Toxicology

Submit Time: 5/3/2010 18:55:22
From: CN=Rose Allison/OU=DC/O=USEPA/C=US
To: CN=Greg Schweer/OU=DC/O=USEPA/C=US@EPA
Cc:
Subject: Re: Fw: Re: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Thanks. Yes, he sends these to me too, so now I know and that Jennifer has handled it. Rose

Rose Allison For Deliveries
Team Leader **EPA East Building**
New Chemicals Program *1201 Constitution Ave NW *
Chemical Control Division (7405M) **Room 4419G**
US EPA **Wash DC 20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

Greg Schweer---05/03/2010 02:51:11 PM---FYI

From: Greg Schweer/DC/USEPA/US

To: Rose Allison/DC/USEPA/US@EPA

Date: 05/03/2010 02:51 PM

Subject: Fw: Re: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

---

FYI

Greg Schweer
Chief, New Chemicals Management Branch
Chemical Control Division
U.S. EPA, Office of Pollution Prevention and Toxics
(202)564-8469

-----Forwarded by Greg Schweer/DC/USEPA/US on 05/03/2010 02:50PM -----

To: Jennifer Seed/DC/USEPA/US@EPA
From: James R Hoover <James.R.Hoover@USA.dupont.com>
Date: 05/03/2010 01:47PM
cc: Greg Schweer/DC/USEPA/US@EPA
Subject: Re: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Jennifer,

Again many thanks, and sorry for the last-minute inconvenience last Friday and today. I think that we did get the message in time this morning to make the needed revisions. Very best regards, Jim

Seed.Jennifer@epamail.epa.gov

05/03/2010 09:10 AM

To James R Hoover/AE/DuPont@DuPont

cc Schweer.Greg@epamail.epa.gov

Subject Re: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Jim,

Sorry about the confusion over this. The protocol is fine. I hope you get this message in time.

Jennifer

Jennifer Seed, PhD
Deputy Director
Risk Assessment Division, OPPT
202-564-7634
seed.jennifer@epa.gov

|---------->
| From: |
|---------->
>----------|
|James R Hoover <James.R.Hoover@USA.dupont.com>
|
>----------|
|---------->
| To: |
|---------->
>----------|
|Jennifer Seed/DC/USEPA/US@EPA
|
>----------|
|---------->
| Date: |
|---------->
>----------|
|04/30/2010 04:16 PM |
>----------|
|---------->
| Subject: |
|---------->
>----------|
|Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -
|

>----------|

Jennifer....Greg Schweer directed me to Jim Allwood, and Jim told me to foward this EMail and give you a quick call, so I will call in a minute. May apologies for this,best rgds, Jim

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 04:14 PM -----

James R
Hoover/AE/DuPont

To
04/30/2010 03:57 PM schweer.greg@epa.gov
cc

Subject
Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Hi Greg....My personal apologies for this very late discovery, and even later communication to Rose and to you, on this issue. This is clearly our mistake, and I take full responsibility for it.

I called Rose to give her a heads-up on the enclosed EMails (below). I now understand from Rose's Voicemail that she is out today.

For us to proceed as indicated, I think we would need a "non-objection" Email from EPA relative to this EMail before 6:00am this coming Monday morning (May 3rd).

The details are show below.

Any advice would be much appreciated. We fully realize this may be difficult to impossible, but I wanted to make fully sure that what ever we do is totally right.

Again, my apologies.

Very best regards, Jim

Jim Hoover, FPS Global Regulatory Manager

DuPONT DCF/FPS
CRP 702, Room 2116
Wilmington, DE 19880

BBerry: Personal Phone / Ex. 6

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 03:40 PM -----

James R
Hoover/AE/D
uPont

To
Allison.Rose@epamail.epa.gov
04/30/2010
cc
03:11 PM Gary W Jepson/AE/DuPont@DuPont, Steven R Frame/AE/DuPont@DuPont, Susan M Munley/AE/DuPont@DuPont, Jane Bradd Andersen/AE/DuPont@DuPont

Subject
Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Hi Rose...Jane is away on vacation, and out of communication range.

My personal, and DuPont company, apologies for this late and urgent 'non-objection' request consideration, but our GenX Toxicity Team has just realized that we overlooked a CRITICAL study design issue in the 18405-1937 Mouse Study Blood Collection Amendment just approved by EPA.

The details are outlined in the Notes from Randy Frame and Sue Munley, shown below.

Given the circumstances and timing, what options do we have to proceed, with EPA agreement, for what Randy and Sue recommend (i.e. a non-objection to proceed).

We fully realize this may be difficult to impossible, but I wanted to make fully sure that what ever we do is totally right.

Many thx for your advice...best regards, Jim

Jim Hoover, FPS Global Regulatory Manager

DuPONT DCF/FPS
CRP 702, Room 2116
Wilmington, DE 19880

BBerry Personal Phone / Ex. 6

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 02:50 PM -----

Steven R
Frame/AE/DuPont

To
04/30/2010 11:46 AM James R Hoover/AE/DuPont@DuPont, Jane Bradd Andersen/AE/DuPont@DuPont
cc
Gary W Jepson/AE/DuPont@DuPont, Susan M Munley/AE/DuPont@DuPont
Subject
URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Jim, Jane,

Please see Sue's note below. In order to get any useful results from the blood collection on adult females in the mouse study, we need to administer a dose on the day of sacrifice (in the current protocol, the last dose is the day before sacrifice). Without a day-of-sacrifice dosing, the blood data from the moms will be of little value. Therefore, it is near certain the EPA would concur with this minor change in procedure since they suggested the blood collection in the first place, and they undoubtedly want the most useful information. Further, this would have no effect on the study results since the animals are sacrificed very soon after the extra dose. Nevertheless, we will need your OK, and EPA's OK to proceed, and we must have this OK before Monday due to the stage of the test we are in. The new amendment could be to the EPA on Monday or soon thereafter.

Randy
----- Forwarded by Steven R Frame/AE/DuPont on 04/30/2010 11:38 AM -----

Susan M
Munley/AE/DuPont

To
04/30/2010 11:37 AM Steven R Frame/AE/DuPont@DuPont, Gary W Jepson/AE/DuPont@DuPont
cc

Subject
18405-1037 mouse study blood collection amendment - URGENT ISSUE

While preparing to execute the blood collection for plasma TK as dictated by the recently-approved protocol amendment 4, we realized that we overlooked a CRITICAL study design issue.

As per protocol, adult animals are scheduled to be dosed through one day PRIOR to scheduled euthanasia.

Based on existing data, blood collection scheduled for two hours following the last dose is the optimal and most meaningful time for collection.

We cannot obtain enough volume for this work without making the bleed a terminal bleed.

Therefore, we need to write another amendment (draft attached below) to specify that animals will be administered a single additional dose on the morning of scheduled euthanasia and then euthanized two hours following that dose.

I am writing to seek non-objection to proceed with this beginning this coming Monday morning, May 3.

The first F0 females to reach PND 21 are scheduled to have their litters weaned and be subsequently euthanized this coming Monday morning.

As these procedures will clarify and improve upon the data dictated by the previously approved amendment 4, please let me know if we can proceed with approving this work to begin on Monday.

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transfers of data to third parties.

Francais Deutsch Italiano Espanol Portugues Japanese Chinese Korean

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(See attached file: 189225 Draft Amendment 5 043010.doc)

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Francais Deutsch Italiano Espanol Portugues Japanese Chinese Korean

http://www.DuPont.com/corp/email_disclaimer.html

[attachment "189225 Draft Amendment 5 043010.doc" deleted by Rick Allison/DC/IS/EM/US]

Subject: Re: Fw: Protocol Review for P08-509
From: CN=Jennifer Seed/OU=DC/O=USEPA/C=US
Submit Time: 12/10/2009 00:36:35
To: CN=Rose Allison/OU=DC/O=USEPA/C=US@EPA CN=Donald Rodier/OU=DC/O=USEPA/C=US@EPA
Cc: CN=Bob Morcock/OU=DC/O=USEPA/C=US@EPA

Sure why not! Do I need to write one of those formal memos? If so who do I address it to and who do I give it to - I do not have CBI lan rights
Rose Allison

----- Original Message -----
From: Rose Allison
Sent: 12/09/2009 04:56 PM EST
To: Jennifer Seed; Donald Rodier
Cc: Bob Morcock
Subject: Re: Fw: Protocol Review for P08-509
Thanks Jennifer. I was speaking with the company contact and they told me that if it could be reviewed this month that they'd get some break on getting it started, so I was wondering if there was any possibility for review by the week of Dec. 21? I thought I'd ask. Rose

---

Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

Yes, do not send this to SRC. If someone can get me the protocol we can get it looked at.

Jennifer Seed, PhD
Deputy Director
Risk Assessment Division, OPPT
202-564-7634
seed.jennifer@epa.gov

Donald Rodier---12/04/2009 11:52:28 AM---Hi Jennifer, Please see Rose's note below. I had sent a mouse reproduction/development study (gavag

From: Donald Rodier/DC/USEPA/US

To: Jennifer Seed/DC/USEPA/US@EPA

Cc: Rose Allison/DC/USEPA/US@EPA, Bob Morcock/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA

Date: 12/04/2009 11:52 AM

Subject: Fw: Protocol Review for P08-509

Hi Jennifer,

Please see Rose's note below. I had sent a mouse reproduction/development study (gavage) to Gordon Cash foe SRC review. Rose mentioned that you or one of your staff would be better suited for the task. Please let me know what you would like to do. I am still learning what I am supposed to be doing.

Don

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

----- Forwarded by Donald Rodier/DC/USEPA/US on 12/04/2009 11:47 AM -----

From: Rose Allison/DC/USEPA/US

To: Donald Rodier/DC/USEPA/US@EPA

Date: 12/04/2009 11:33 AM

Subject: Re: Protocol Review for P08-509

Please ask Jennifer. I'd prefer that she is involved so either she or someone on he staff should do it, I think. Rose

Rose Allison
202/564-8970/FAX 202/564-9490

Donald Rodier---12/04/2009 10:56:33 AM---Hi, I will check with Jennifer about the protocols for this test. Is it alright for SRC to start re

From: Donald Rodier/DC/USEPA/US

To: Rose Allison/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA, Jennifer Seed/DC/USEPA/US@EPA

Date: 12/04/2009 10:56 AM

Subject: Re: Protocol Review for P08-509

Hi,

I will check with Jennifer about the protocols for this test. Is it alright for SRC to start reviewing it or do you believe that one of Jennifer's staff should do it?

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

Rose Allison---12/04/2009 10:20:29 AM---Don, Jennifer has been involved in these protocol reviews and in my mind should be consulted. This

From: Rose Allison/DC/USEPA/US

To: Donald Rodier/DC/USEPA/US@EPA, Gordon Cash/DC/USEPA/US@EPA

Cc: Bob Morcock/DC/USEPA/US@EPA

Date: 12/04/2009 10:20 AM

Subject: Protocol Review for P08-509

Don, Jennifer has been involved in these protocol reviews and in my mind should be consulted. This is a protocol that has specific modifications that we required and we've allowed some deviation based on ORD's more recent work. Rose.

Rose Allison
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

Hi Gordon,

You may have already seen Oscars note about my handling past due PMN cases. I am already getting actions. This email is about a 5e order. We are supposed to review a protocol for an oral (gavage) reproduction/development study with mice. The PMN is P-08-509 and the DCO number is 5010000683. Would you please have SRC review this protocol. Although the due date is January 5, we need time for a QA/QC and I don't have a clue who will do this, so could you ask SRC to complete it by Dec.23? You can tell I am new at this so if I have omitted anything please let me know.

Don

Donald Rodier, Chief
Science Support Branch
Risk Assessment Division/OPPT
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Mail Code 7403M
Washington, DC 20460
phone: 202-564-7633
fax: 202-564-7450

From: CN=Rose Allison/OU=DC/O=USEPA/C=US
To:
Cc:
Subject: Fw: TSCA 8e Letter - P-08-509

This is the 8(e) that will be discussed in the meeting currently scheduled for Wed., April 7 at 11:00 am in 4140.. Scroll down to the bottom for the attachment

---

Rose Allison
Team Leader
New Chemicals Program
Chemical Control Division (7405M)
US EPA
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For Deliveries
EPA East Building
*1201 Constitution Ave NW
Room 4419H
Wash DC 20004

----- Forwarded by Rose Allison/DC/USEPA/US on 03/18/2010 04:31 PM -----

From: Mike Kaplan <Mike.Kaplan@USA.dupont.com>

To: Rose Allison/DC/USEPA/US@EPA

Cc: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>, James R Hoover <James.R.Hoover@USA.dupont.com>, Mike Kaplan <Mike.Kaplan@USA.dupont.com>

Date: 02/23/2010 08:36 AM

Subject: TSCA 8e Letter - P-08-509

Dear Rose Allison,

Per Jane Bradd Andersen's request, attached is a copy of the letter for P-08-509.

Sincerely,

Mike

A.Michael Kaplan, Ph.D.
Director - Regulatory Affairs
DuPont Haskell Global Centers for Health & Environmental Sciences
1090 Elkton Road
P.O. Box 50
Newark, DE 19714
302-366-5260 Phone
302-451-4531 Fax
mike.kaplan@usa.dupont.com

(See attached file: 2-5-10 FRD-902 2010-033 Letter.pdf)

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2-5-10 FRD-902 2010-033 Letter.pdf 2-5-10 FRD-902 2010-033 Letter.pdf

PBI / Ex. 4

February 5, 2010

Via Federal Express

Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency, ICC Building
1201 Constitution Ave., NW
Washington, DC 20004

Dear 8(e) Coordinator:

PBI / Ex. 4

This letter is to inform you of the preliminary results of a developmental toxicity study in rats with the above referenced test substance. This test substance is subject to a Consent Order, PMN P-08-509.

Groups of 22 time-mated Crl:CD(SD) rats were administered solutions of the test substance in deionized water at dose levels of 0, 10, 100, or 1000 mg/kg/day. Dosing was initiated on gestation day (GD) 6 and continued through GD 20. During the in-life portion of the study, maternal body weights and food consumption as well as clinical observations data were collected. On GD 21, dams were euthanized and underwent a gross external and internal examination. Weights for maternal livers and kidneys were recorded and these tissues were preserved for future histopathologic examination. The gravid uteri were removed, weighed, and dissected. Uterine contents were described and fetuses were counted, weighed, sexed, and examined for external, visceral, head, and skeletal alterations.

There was a dose-related increase in the number of dams found with early deliveries in their cages on the morning of GD 21. There were 0, 0, 4, and 9 dams found delivered at 0, 10, 100, and 1000 mg/kg/day, respectively. In addition, mean fetal weight was 8 and 28% lower than controls at 100 and 1000 mg/kg/day, respectively; these reductions were statistically significant. Slight reductions in maternal body weight and food consumption occurred at 1000 mg/kg/day. Maternal kidney weights were significantly higher at 1000 mg/kg/day and maternal liver weights were significantly higher at 100 and 1000 mg/kg/day. The remaining data collected to date were generally comparable to control group data across all groups tested. There were no test substance-related increased in fetal resorptions, malformations, or variations at any dose level tested. Maternal histopathology examinations are currently in progress.

This information is submitted in accordance with current guidance issued by EPA indicating EPA's interpretation of Section 8(e) of the Toxic Substances Control Act or, where it is not clear that reporting criteria have been met, it is submitted as a precautionary measure and because it is information in which EPA may have an interest.

Sincerely,

PBI / Ex. 4

From: PBI / Ex. 4 @USA.dupont.com>
Cc: @USA.dupont.com> James R Hoover <James.R.Hoover@USA.dupont.com>
To: Rose Allison/DC/USEPA/US@EPA
Subject: 2 Year Study Protocol Revisions
Submit Time: 7/2/2010 19:20:14

Dear Rose:

Thank you so much for your guidance this afternoon. Jim and I certainly appreciated it.

Attached is the revised 2 year study protocol we discussed (with the edits in tracked mode) for the Agency's review and approval.

The changes from what was sent to EPA previously are:

The study director and alternate contact people have changed (due to change in work assignment of the originally assigned study director)

An additional 10 animals per group were added to each group to bring the number designated for carcinogenicity evaluation from 60 to 70 per group. This was done because of concerns about survival due to the requirement to ad lib feed.

While we were waiting for EPA's approval, the lab conducted the analytical method validation, including verifying stability of the test substance in the diet. Therefore, this work does not need to be repeated on samples from diets prepared for the study and the references to stability evaluation were removed from the protocol (the validation work was referenced to support this).

A few changes were made to their sentinel animal health evaluation procedures.

The study is scheduled to begin on July 29. Let me or Jim Hoover know if you have any questions.

Best regards,

PBI / Ex. 4
DuPont Chemicals and Fluoroproducts
Wilmington, Delaware
PBI / Ex. 4

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125-141 protocol 6_28_10 sam comments.doc 

H-28548: Combined Chronic Toxicity/Oncogenicity Study
2-Year Oral Gavage Study in Rats

Work Request Number 18405

Service Code 1238

DuPont Report Number – 18405-1238

Protocol

June 28, 2010

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ED_002003A_00067467-00003

1. INTRODUCTION

1.1. Study Number

DuPont Work Request/Study Code Number: DuPont-18405/1238

DuPont Report Number: 18405-1238

MPI Research Study Number: 125-141

1.2. Study Title

H-28548: Combined Chronic Toxicity/Oncogenicity Study 2-Year Oral Gavage Study in Rats

1.3. Sponsor

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898, U.S.A.

1.4. Sponsor Representative

Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T.
Senior Research Toxicologist
DuPont Haskell Global Centers for Health and Environmental Sciences
P.O. Box 50
Newark, Delaware 19714 U.S.A.

Telephone: 302-366-6389
Telefax: 302-366-5211
E-mail: Susan.A.MacKenzie@USA.dupont.com

1.5. Objective

The objective of this study is to evaluate the potential chronic toxicity and oncogenicity of H-28548 when administered via oral gavage over the major portion of the life span of the test animals.

1.6. Regulatory Guideline

This protocol meets the United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Guideline 870.4300, Combined chronic toxicity/carcinogenicity, August 1998. The experimental design and methods are also based on the Organization for Economic Cooperation and Development (OECD) Guideline 453, September 2009, the Japanese Ministry of Agriculture, Forestry and Fisheries Guidelines for Data Requirements for Supporting Registration of Pesticides, No. 12-Nousan-8147, Notification by Director-General dated 24 November, 2000, and the Commission Directive 88/302/EEC B.33 Combined Chronic/Carcinogenicity test, *Methods for the Determination of Toxicity* (1988).

ED_002003A_00067467-00004

1.7. Good Laboratory Practice

This nonclinical laboratory study will be conducted in accordance with the United States Environmental Protection Agency FIFRA Good Laboratory Practice (GLP) Standards, 40 CFR Part 160, Toxic Substance Control Act Good Laboratory Practice Standards, 40 CFR Part 792, the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice ENV/MC/CHEM(98)17, and the Japanese Good Laboratory Practice Standards, 11 Nohsan No. 6283 and as changed in 12 Nohsan No. 8628, and 13 Seisan No. 1660.

1.8. Testing Facility

MPI Research, Inc.
54943 North Main Street
Mattawan, MI 49071-9399 U.S.A.

MPI Research is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

1.9. Computer Systems

The following are the proposed computer systems to be used during the conduct of this study. The actual systems used will be documented in the final report.

MPI Research Computer Systems	
Provantis™:	Client-server, Oracle-based system primarily used for toxicology studies.
Niagara Framework® Software System or Siemens Environmental Monitoring System (EMS):	Environmental monitoring, alarming, and reporting application.
Dispense:	Automates the test article control processes.
Microsoft® Windows XP:	Used in conjunction with Empower 2 software
Empower 2:	Empower 2 Chromatographic Data System used to quantitatively determine the amounts of analytes in samples, including test articles in formulation.
MPI Archiving System (MArcS):	In-house developed application for automated storage and retrieval information for archiveable materials (e.g. lab books, study data, wet tissues, slides, etc.).
Enterprise Reporting System – Table Production System (TPS):	In-house developed reporting system used primarily for reporting of Provantis™ data.
Master Schedule:	Maintains the master schedule for the company.

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SAS®:	The SAS® System is an integrated system of software products that enables a user to perform data entry, retrieval, data management, reporting, graphics, statistical analysis, and applications development.
Microsoft® Office 2003 Professional:	Bundle of integrated productivity tools including word and data processing and communications software. Contains the utilities Microsoft® Access, Excel, InfoPath, Outlook, PowerPoint, Publisher, and Word.
docuBridge®:	Electronic publishing system.

1.10. Personnel

1.10.1. Study Director

Lisa Craig, B.S.

Telephone: 269-668-3336 ext. 1367
Telefax: 269-668-4151
E-mail: lisa.craig@mpiresearch.com

1.10.2. Alternate Contact

Chris N. Papagiannis, B.S.

Telephone: 269-668-3336 ext. 1392
Telefax: 269-668-4151
E-mail: chris.papagiannis@mpiresearch.com

1.11. Proposed Study Schedule

Study Initiation Date (EPA and OECD): (Date Study Director signs Study Approval-Initiation Line in the protocol)	Date Study Director signs Study Approval-Initiation Line in this protocol
Experimental Starting Date (OECD): (Date of the first data collection directly from the study)	To be added by amendment.
Experimental Start Date (EPA): (Date of first test article exposure)	To be added by amendment.
Experimental End Date (EPA): (Date of last animal termination)	To be added by amendment.
Experimental Completion Date (OECD): (Date of the last data collection directly from the study)	Date Anatomic Pathology Contributor report is signed
Draft Report Mail Date:	To be added by amendment

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1.12. Quality Assurance

This study will be subjected to periodic inspections and the data, draft and final reports will be reviewed by the Quality Assurance Department of MPI Research in accordance with MPI Research's Standard Operating Procedures. Study quality assurance inspection records will be made available to the Sponsor Representatives during visits to MPI Research.

1.13. Alteration of Design

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, MPI Research will honor such change. However, written authorization will be obtained thereafter. All protocol amendments and justifications will be documented, signed, and dated by the Study Director and Sponsor. The protocol and all amendments will be issued to the Sponsor as well as at MPI Research.

1.14. Declaration of Intent

This study may be submitted to an Organization for Economic Cooperation and Development (OECD) member country, the United States Environmental Protection Agency (EPA), and/or other country regulatory bodies.

2. TEST AND CONTROL ARTICLES

2.1. Description of Test Article

2.1.1. Identity

HFPO Dimer Acid Ammonium Salt (aka H-28548)

Haskell number: 28548

R&D Lot Number: E109540-44A

A description, lot number, storage conditions, expiration date, safe handling procedures, physical properties, as well as other relevant information will be documented in the study data.

2.1.2. Test Article Properties

The Sponsor will provide a certificate of analysis (COA) documentation on the purity, composition, stability, and other pertinent information, unless otherwise noted.

2.2. Test Article Preparation

The bulk test article will be stored at room temperature. The test article formulations will be adjusted for a purity of 84%. The test article will be mixed with deionized water to achieve the desired dose volumes. The vehicle and method of preparation will be determined based upon physical characteristics of the test article and size of batches required. Fresh formulations will be prepared for each concentration weekly and stored ambient when not in use.

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2.3. Test Article Analysis

Test article formulations prepared for the study will be evaluated for homogeneity and concentration. Room temperature stability (at least 14 days) which covers the concentration range to be used in this study has been established in MPI Research Study Number 125-128. No further stability analysis is necessary.

Appropriate samples (see table below) will be taken while the preparations are stirring. Homogeneity will be evaluated again if the batch size changes by more than 50% during the study or if a new concentration is outside of the range of concentrations previously evaluated. Following acceptance of the analytical results (signing of the final report) by the Study Director, or at the Study Director's discretion, backup samples will be discarded.

Analytical Sample Collection Table

Sample Type	Concentrations to Sample	Stratum	Number of Samples per Concentration			Sample Volume (mL)	Intervals
			Collected	Analyzed	Back up		
Homogeneity Analyses[a]	Low and high	Top Middle Bottom	6 6 6	2 2 2	4 4 4	1 1 1	Week 1
Concentration Analyses[a]	All (including control)	Middle	6	2	4	1	Weeks 1-4, every 3 months thereafter

[a]: *The samples will be stored frozen at approximately -20 °C pending analyses or final disposition.*

2.4. Analyses

All analytical work will be conducted by MPI Research, Inc., State College, PA using an analytical method developed by MPI Research and validated under MPI Research Study Number 125-128. The work performed in conjunction with this study will be conducted in compliance with GLPs and subject to review by the Quality Assurance Unit (QAU) of that laboratory. The findings of their QAU will be submitted to the Principal Investigator and the Principal Investigator's Management as well as to the MPI Research Study Director and MPI Research Management. A final report, including a Quality Assurance Statement, will be prepared and submitted to MPI Research for inclusion as an appendix in the main study final report. Samples will be shipped on dry ice on Monday through Wednesday for next day delivery. The primary contact will be notified prior to each shipment.

Principal Investigator (Formulation Analyses)	Primary Contact for Sample Shipment
Sharon Lupo 3058 Research Drive State College, PA 16801 Telephone: 814-272-1039	Attn: Sample Control 3048 Research Drive State College, PA 16801 Telephone: 814-272-1039

ED_002003A_00067467-00008

Telefax: 814-231-1580 E-mail: Sharon.lupo@mpiresearch.com	Telefax: 814-231-1580 E-mail: SC1SampleReceiving@mpiresearch.com

2.5. Reserve Sample

A reserve sample from each batch of test article used in this study will be collected and stored at MPI Research in a secure area with the appropriate environmental controls. If multiple studies are conducted with the same test article, a common reserve sample may be taken and labeled appropriately.

2.6. Test Article Disposition

Any remaining test article will be returned to the Sponsor after completion of the study. The test article will be shipped to the following address:

Brett Cordrey
E.I. du Pont de Nemours and Company
Stine Haskell Research Center
Building S120/103
1090 Elkton Road
Newark, DE 19714
Telephone: 302-366-5542

Mr. Cordrey will be notified prior to shipment.

2.7. Description of Vehicle

2.7.1. Identity

Deionized water

A description, lot number, storage conditions, expiration date, safe handling procedures, physical properties, as well as other relevant information will be documented in the study data.

2.7.2. Vehicle/Control Article Properties

The vehicle used will be water distilled from deionized tap water at the Testing Facility.

3. TEST SYSTEM

3.1. Species

Rat

3.2. Strain

CD® [Crl:CD(SD)]

3.3. Source

Charles River Laboratories

ED_002003A_00067467-00009

3.4. Justification of Test System

The current state of scientific knowledge and the applicable guidelines cited previously in this protocol do not provide acceptable alternatives, *in vitro* or otherwise, to the use of live animals to accomplish the purpose of this study. "The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species."[1] "Whole animals are essential in research and testing because they best reflect the dynamic interactions between the various cells, tissues, and organs comprising the human body."[2]

The rat is a frequently used model for evaluating the toxicity of various classes of chemicals and for which there is a large historical database.

3.5. Expected Age

The test animals will be approximately 4-5 weeks of age at arrival. All animals placed on study will be less than 8 weeks of age at the start of dosing.

3.6. Expected Body Weight

The males will weigh approximately 100 to 125 g and the females will weigh approximately 76 to 100 g at arrival, as measured within 3 days of arrival. The actual range may vary but will be documented in the data.

3.7. Number of Animals

3.7.1. Number Ordered

Males: 400
Females: 400

3.7.2. Number on Study (includes 25 sentinel animals per sex)

Males: 345
Females: 345

Females will be nulliparous and non-pregnant.

3.7.3. Justification for Number on Study

This study was designed to use the fewest number of animals possible, consistent with the objective of the study, the scientific needs of the Sponsor, contemporary scientific standards, and in consideration of applicable regulatory requirements cited previously in this protocol. This study is designed to use the smallest number of animals possible that will allow sufficient group sizes for meaningful statistical analysis of data.

[1] "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training", Federal Register, 1985 May 20; 50(97).
[2] "Position Statement on the Use of Animals in Research", 1993 Feb 26; NIH Guide 22(8).

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3.7.4. Selection for Study

All animals placed on study will have body weights that fall within ±20% of the mean body weight for each sex. If not enough animals fall within this weight range to satisfy the number of animals required to go on study, the Study Director will be notified to ascertain the appropriate action to be taken.

Animals considered suitable for study will be weighed prior to treatment. After the appropriate number of animals with the highest and lowest body weights has been excluded, the remaining required number of animals on study will be randomized, by sex, into treatment groups using a standard, by weight, measured value randomization procedure.

3.7.5. Method of Identification

Each animal will be assigned an animal number to be used in Provantis™ and will be implanted with a microchip bearing a unique identification number. The individual animal number, implant number, and the MPI Research study number will comprise a unique identification for each animal. The animal cage will be identified by the study number, animal number, group number, and sex.

3.8. Husbandry

3.8.1. Acclimation

All animals will be permitted an acclimation period of approximately 2 weeks. During this acclimation period, all animals will be observed daily for any clinical signs of disease and all animals will be given a detailed clinical examination prior to selection for study. All animals with any evidence of disease or physical abnormalities will not be selected for study. The week prior to dose initiation, animals will be administered a sham dose of tap water on at least 2 occasions in the same manner and at the same volume intended for use during the study period.

3.8.2. Housing

The animals will be pair-housed (same sex) in solid-bottom cages (polyboxes). In order to foster the rat's natural chewing instinct and keep their teeth at a healthy length, approved chew toys (e.g. Nylabone) will be offered.

3.8.3. Environmental Conditions

Fluorescent lighting will be provided via an automatic timer for approximately 12 hours per day. On occasion, the dark cycle may be interrupted intermittently due to study-related activities. Temperature and humidity will be monitored and recorded daily and maintained to the maximum extent possible between 64 to 79° F and 30 to 70%, respectively.

3.8.4. Diet and Drinking Water

3.8.4.1. Basal Diet

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The basal diet will be block Lab Diet® Certified Rodent Diet #5002, PMI Nutrition International, Inc. This diet will be available *ad libitum* unless designated otherwise. Each lot number used will be identified in the study records.

3.8.4.2. Basal Diet Contaminants

The Study Director is not aware of any potential contaminants likely to be present in the certified diet that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier will be conducted.

3.8.4.3. Water

Tap water will be supplied *ad libitum* via an automatic water system unless otherwise indicated.

3.8.4.4. Water Contaminants

The drinking water used will be monitored for specified contaminants at periodic intervals according to MPI Research Standard Operating Procedures. The Study Director is not aware of any potential contaminants likely to be present in the water that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

3.9. Sentinel Animals

A health screen will be conducted pretest and at 6, 12, 18, and 24 months on 3-5 males and 3-5 females (depending on survival) using sentinel animals selected with a computerized randomization and euthanized via carbon dioxide inhalation for this purpose. If insufficient animals are available due to survival, fewer animals may be submitted for evaluation (Study Director consulted) and this will be noted in the final report. Approximately 1-2 mL of blood will be collected via the vena cava and serum obtained. Blood samples will be processed to serum and placed into 2 aliquots of approximate equal volume. Serum samples will be stored at approximately -20°C. A gross necropsy will be performed at the time of blood collection. Gross lesions will be recorded. No tissues will be saved. Any sentinel animal that is found dead or euthanized *in extremis* will receive a gross necropsy and gross lesions will be saved for possible histopathologic evaluation.

The serum will be evaluated as indicated below:

3.9.1. Pretest and at months 12 and 24

- Pneumonia Virus
- Reovirus Type 3
- Theiler's Encephalomyelitis Virus (GD-7)
- Lymphocytic Choriomeningitis Virus
- Sendai Virus
- Mycoplasma Pulmonis
- Kilham Rat Virus

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- Rat Coronavirus/Sialodacryoadenitis Virus
- Toolan's H-1 Virus
- Rat Parvovirus

3.9.2. At months 6 and 18

- Sendai Virus
- Kilham Rat Virus
- Rat Coronavirus/Sialodacryoadenitis Virus
- Toolan's H-1 Virus
- Rat Parvovirus
- Mycoplasma Pulmonis

Initial testing will be performed at MPI Research. For positive or inconclusive results, confirmation testing will be performed by BioReliance. Samples will be sent at ambient temperature to the following address, if necessary.

Principal Investigator: Dr. Arlene Leon
BioReliance Corporation

9900 Blackwell Road
Rockville, MD 20850

Telephone: 301-610-2641
Telefax: 301-610-2587

Any actions based on the results of the health screen will be determined after consultation with the Sponsor. Testing will not be conducted in accordance with GLPs. This will be included as a GLP exception in the final report. Results of these analyses will be maintained in the study file.

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4. STUDY DESIGN

GROUP	Dose Level (mg/kg/day)	Number of Animals: Initial M	Initial F	Clinical Pathology[a] M	Clinical Pathology[a] F	12-Month Interim Necropsy[a, b] M	12-Month Interim Necropsy[a, b] F	Terminal Necropsy M	Terminal Necropsy F	Microscopic Pathology[c] M	Microscopic Pathology[c] F
1	0	80	80	10	10	10	10	70	70	80	80
2	0.1	80	-	10	-	10	-	70	-	AR	-
3	1	80	80	10	10	10	10	70	70	AR	AR
4	50	80	80	10	10	10	10	70	70	80	AR
5	500	-	80	-	10	-	10	-	70	-	80
89*	-	25	25	-	-	-	-	-	-	-	-

a: Hematology, and clinical chemistry will be performed on 10 animals/sex/group at 3 months. Hematology, coagulation, clinical chemistry, and urinalysis evaluations will be conducted on 10 animals/sex/group at 6 and 12 months. A differential blood smear will be prepared on all animals designated for necropsy at 12 months, all survivors at12, 18, and 24 months(termination), and all animals euthanized in extremis.
b: An interim necropsy will be conducted at 12 months on 10 animals/sex/group.
c: Animals from both the 12 month interim and terminal necropsies, and other animals as required..
AR = As Required: 1) Target tissues identified by high dose group evaluations, 2) Tissues in all animals found dead or euthanized in a moribund condition, and 3) gross lesions.
**Sentinel animals*

5. TEST AND CONTROL ARTICLE ADMINISTRATION

5.1. Route of Administration

The test and control articles will be administered by gavage.

5.2. Justification for Route of Administration and Dose Selection

The oral gavage route was selected as the most efficient way to administer an accurate dose.

In a previous study (DuPont-17751-1026), Crl:CD(SD) rats (10/sex/dose) were dosed with the test substance by oral gavage for at least 90 days at daily doses of 0, 0.1, 10, or 100 mg/kg/day for males and 0, 10, 100, or 1000 mg/kg/day for females. In the 1000 mg/kg/day group, three females died prior to scheduled sacrifice and others displayed clinical signs. No other test substance-related effects were observed in surviving animals in all groups on body weight or nutritional parameters, clinical or ophthalmological observations, or neurobehavioral parameters.

Test substance-related findings included regenerative anemia (males: 100 mg/kg/day; females: 1000 mg/kg/day), clinical chemistry effects consistent with PPARα activation (males: ≥10 mg/kg/day; females: 100-1000 mg/kg/day), and increased liver weights and associated hepatocellular hypertrophy (males: ≥10 mg/kg/day; females: 1000 mg/kg/day). Similar liver effects were observed at ≥3 mg/kg/day in males and 300 mg/kg/day in females in a rat 28-day gavage study (DuPont-24447). Increased kidney weights were observed in males and females at ≥10 mg/kg/day. In females, renal papillary necrosis and/or renal tubular necrosis were

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observed in the two females found dead prior to scheduled sacrifice and in one female that survived to scheduled sacrifice. Clinical and anatomic pathology parameters were fully or partially (male hematology effects; liver weights) reversible after an approximate 4-week recovery period.

Based on the results of the 90-day and 28-day studies, doses selected for this study were 0, 0.1, 1, and 50 mg/kg/day in males and 1, 50, and 500 mg/kg/day in females. The high dose is expected to produce effects on clinical chemistry and liver weight and microscopic pathology in males and females, without producing excessive liver toxicity. The middle dose may produce liver and clinical chemistry in either sex but could be a no-observed-adverse-effect level (NOAEL). The low dose is expected to be a NOAEL in both males and females.

5.3. Frequency and Duration of Administration

The test and control articles will be administered once per day, at approximately the same time of day (i.e., if the Day 1 dose occurs in the am, then subsequent doses should be delivered in the am for the study duration), for at least 104 weeks. The animals will be dosed up to the day prior to scheduled necropsy.

5.4. Dose Volume

10 mL/kg/dose

5.5. Test Article Administration

For administration, the test and control articles will be dosed via oral gavage in accordance with SOP TMA-1. The control animals will receive the control article at the same volume as the test article. Individual doses will be based on the most recent body weights.

6. ANTEMORTEM STUDY EVALUATIONS

6.1. Ophthalmoscopic Examinations

All animals in all groups will be examined prior to exposure and all surviving animals prior to the scheduled necropsy (interim and terminal) in accordance with SOP TOX-61. The ophthalmological examinations will be conducted by a veterinary ophthalmologist.

6.2. Cageside Observations

All animals will be observed at least twice a day for morbidity, mortality, injury, and availability of food and water in accordance with SOP ACU-65. The afternoon cageside observation will be conducted at the same approximate time of day (± 2 hours). Beginning on Week 53, a third mortality check in the evening will also be conducted. Any animals in poor health will be identified for further monitoring and possible euthanasia.

Any abnormal findings noted in the morning cageside observation will be recorded by exception (i.e., 'no abnormalities detected' will not be captured on a daily basis for every animal).

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6.3. Detailed Clinical Examinations

A detailed clinical examination of each animal will be performed once during each study week in accordance with SOP TOX-2. Observations will include, but will not be limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, bizarre behavior, and palpation of tissue masses in accordance with SOP TOX-3.

6.4. Body Weights

Body weights will be measured and recorded within 3 days of arrival, at least once prior to randomization, weekly during the first 13 weeks starting on Day 1 (prior to dosing), and every other week thereafter in accordance with SOP TOX-4. The individual and mean group mean body weights gain will be calculated and reported weekly (starting on Week -1), for the first quarter (Weeks 1-13), the first year (Weeks 1-52), and the entire study (Weeks 1-104).

6.5. Food Consumption

Food consumption will be measured and recorded pretest (Week -1), weekly during the first 13 weeks, and for 2 weeks intervals starting at Week 14 (i.e., food consumption will represent a 14 day interval) in accordance with SOP TOX-5. Food consumption will be measured for the cage and divided by the number of surviving animals. The individual and mean group mean food consumption and food efficiency will be calculated and reported weekly (starting on Week -1), for the first quarter (Weeks 1-13), the first year (Weeks 1-52), and the entire study (Weeks 1-104).

6.6. Clinical Pathology

The animals will have free access to drinking water but will be fasted overnight (no more than 16 hours) prior to sample collection. Blood samples (approximately 3 mL) taken at non-terminal intervals will be taken via the via the sublingual vein. Blood samples (3-5 mL) taken at necropsy, including those animals euthanized *in extremis* will be taken via the vena cava. Where possible, the animals designated for clinical pathology evaluations at 3 and 6 months will be the same animals evaluated at 12 months. The order of bleeding and analysis will be alternating (one animal from each dose group, then repeating) to reduce handling and time biases. If samples need to be recollected for hematology, coagulation, or urinalysis for sample quality purposes (e.g., clotted sample), animals do not need to be fasted.

The following clinical pathology tests will be conducted.

6.7. Hematology

6.7.1.1. Number of Animals

10/sex/group at 3, 6 and 12 months (Animals designated for chronic toxicity evaluation)

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6.7.1.2. Collection Intervals

3, 6, and 12 months

6.7.1.3. Parameters Evaluated

- leukocyte count (total and absolute differential)
- erythrocyte count
- hemoglobin
- hematocrit
- mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated)
- absolute reticulocytes
- platelet count
- blood cell morphology

6.7.2. Coagulation

6.7.2.1. Number of Animals

10/sex/group at 6 and 12 months (Animals designated for chronic toxicity evaluation)

6.7.2.2. Collection Intervals

6 and 12 months

6.7.2.3. Parameters Evaluated

- prothrombin time
- activated partial thromboplastin time

6.7.3. Clinical Chemistry

6.7.3.1. Number of Animals

10/sex/group at 3, 6, and 12 months (Animals designated for chronic toxicity evaluation)

6.7.3.2. Collection Intervals

3, 6, and 12 months

6.7.3.3. Parameters Evaluated

- alanine aminotransferase
- alkaline phosphatase
- sorbitol dehydrogenase
- total protein
- albumin
- globulin and A/G (albumin/globulin) ratio (calculated)
- urea nitrogen
- creatinine

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- total cholesterol
- triglycerides
- total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dl)
- aspartate aminotransferase
- total bile acids
- glucose
- calcium
- phosphorus
- electrolytes (sodium, potassium, and chloride
- gamma glutamyl transferase

6.7.4. Urinalysis

Animals will be placed in stainless steel metabolism cages for at least 12 hours to collect urine.

6.7.4.1. Number of Animals

10/sex/group at 6 and 12 months (Animals designated for chronic toxicity evaluation)

6.7.4.2. Collection Intervals

6 and 12 months

6.7.4.3. Parameters Evaluated

- volume
- specific gravity
- pH
- color and appearance
- protein
- glucose
- bilirubin
- ketones
- blood
- urobilinogen
- microscopy of centrifuged sediment

6.7.5. Peripheral Blood Smears

6.7.5.1. Number of Animals

All surviving animals (animals designated for carcinogenicity evaluation) and animals euthanized *in extremis*

6.7.5.2. Collection Intervals

12 and 18 months and prior to termination (24 months)

Peripheral blood smears will be prepared and held for possible future analysis from all surviving animals at 12, 18, and 24 months (study termination). The total and differential

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leukocyte count will be made on those animals in the control and highest dose group (Groups 1 and 4 or 5) at termination. If these data, or data from the pathology examination, indicate a need, then the blood smears from the other dose groups and/or earlier time point will also be examined. If clinical observations suggest a deterioration of health of the animals during the study, a differential blood count of the affected animals will be performed.

7. EUTHANASIA

7.1. Moribundity

Any moribund animals, as defined by a Testing Facility Standard Operating Procedure (ACU-47), will be euthanized for humane reasons and to prevent the loss of tissues through autolysis. All animals euthanized *in extremis* or found dead will be subjected to a routine necropsy. Where practical, a full set of tissues as listed in the Postmortem Study Evaluations portion of this protocol will be collected and preserved in the appropriate fixative.

7.2. Method of Euthanasia

Euthanasia will be by carbon dioxide inhalation followed by a MPI Research SOP (NEC-12) approved method to ensure death, e.g. exsanguination.

7.3. Final Disposition

All surviving animals placed on study will be euthanized at their scheduled necropsy or, if necessary, euthanized *in extremis*. Extra animals obtained for this study, but not placed on study, will be transferred to either an MPI Research stock or training colony, or euthanized and discarded. The final disposition of each animal will be documented in the study records.

8. POSTMORTEM STUDY EVALUATIONS

Complete necropsy examinations will be performed under procedures approved by a veterinary pathologist on all animals dying spontaneously, euthanized *in extremis*, or euthanized at scheduled necropsies in accordance with SOP NEC-42. Examinations will be performed 7 days a week. Animals that are found dead after regular working hours will be refrigerated overnight and necropsies performed at the start of the next working day. At the appropriate intervals (after 12 and 24 months), all appropriate animals will be euthanized and examined.

The animals will be examined carefully for external abnormalities including palpable masses. The skin will be reflected from a ventral midline incision, and any subcutaneous masses will be identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities will be examined for abnormalities and the organs removed, examined, and, where required, placed in fixative. The pituitary will be fixed *in situ*. The eyes and testes will be

[3] Latendresse JR, Warbrittion AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol. 2002 Jul-Aug;30(4):524-33.

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fixed using a modified Davidson's fixative[3]. All other tissues will be fixed in neutral buffered formalin. Formalin will be infused into the lung via the trachea and into the urinary bladder.

Body weight and the organ weights identified in the following table will be recorded for all animals at scheduled necropsies and appropriate organ weight ratios will be calculated (relative to body and brain weights). Paired organs will be weighed together. A combined weight of the thyroid gland with the bilateral parathyroid post fixation will be obtained. Organs will not be weighed for animals dying spontaneously or euthanized *in extremis*.

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections will be performed on sections of tissues and from the groups identified in the following table and all animals dying spontaneously or euthanized *in extremis*.

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination (Groups)[a]	
			1, 4/5	2-3/4
Adrenal gland	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear[b]		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	
Coagulating gland		X	X	
Epididymis	X	X	X	
Esophagus		X	X	
Eye (with retina and optic nerve)		X	X	
GALT[c]		X	X	
Harderian gland		X	X	
Heart	X	X	X	
Joint, tibiofemoral		X	X	
Kidney	X	X	X	

[3] Latendresse JR, Warbrittion AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol. 2002 Jul-Aug;30(4):524-33.

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Lacrimal gland, exorbital		X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver	X	X	X	
Lung with bronchi		X	X	
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Mammary gland (process females only)		X	X	
Nerve, sciatic		X	X	
Nose (4 sections)		X	X	
Ovary with oviduct	X	X	X	
Pancreas		X	X	
Pharynx		X	X	
Pituitary		X	X	
Prostate		X	X	
Salivary gland, mandibular		X	X	
Salivary gland, parotid		X	X	
Salivary gland, sublingual		X	X	
Seminal vesicles		X	X	
Skeletal muscle, biceps femoris		X	X	
Skin		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, lumbar		X	X	
Spinal cord, thoracic		X	X	
Spleen	X	X	X	
Stomach, glandular		X	X	
Stomach, nonglandular		X	X	
Target Organs[d]		X	X	X
Testis	X	X	X	

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Thymus		X	X	
Thyroid gland (with parathyroid)[e]	X	X	X	
Tongue		X	X	
Trachea		X	X	
Ureters		X	X	
Urinary bladder		X	X	
Uterus with cervix	X	X	X	
Vagina		X	X	
Gross lesions		X	X	X
Tissue masses with regional lymph node[f]		X	X	X

[a] *Microscopic examination will be conducted in controls and in Group 4 males and Group 5 females, the respective high dose for each sex.*

[b] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).*

[c] *Gut associated lymphoid tissue*

[d] *Target organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).*

[e] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*

[f] *A regional lymph node drains the region where a tissue mass is located.*

The presence of test article-related lesions in animals from the high dose group will require microscopic examination of the affected target tissue(s) in all animals from the lower dose groups. If mortality in the high dose groups is sufficiently high to preclude assessment of a potential toxic response, all protocol-required tissues from all animals in the next lower dose group will be examined after consultation with the Sponsor (additional cost).

The pathologist may use special stains and techniques as needed to aid in the diagnosis of specific lesions. If after routine sectioning, a tissue is missed, the block will be resectioned once or the tissue re-embedded for resectioning. If the tissue is still missing, the block will not be resectioned unless the missing tissue is determined to be a target organ. In this case, the tissue will be resectioned until located or until it is determined that it is not present in the block or in wet tissue. All missing tissues will be identified in the pathology portion of the final report. Tissues that are unintentionally sectioned or present in the plane with a required tissue, though not required by protocol, will be examined and documented, if abnormal.

A pathologist other than the study pathologist will perform a formal peer review of the histopathologic findings. This review will consist of an examination of all tissues determined to be target organs by the study pathologist, all neoplasms diagnosed in the study and all

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tissues from 10% of the animals selected randomly from control and high dose groups. Other selected tissues may be examined at the discretion of the reviewing pathologist. A signed statement by the reviewing pathologist will appear in the final report.

9. STATISTICS

The following is the proposed analysis plan to be used when data assumptions are met. If there are deviations to this plan due to violations of assumptions or if any other techniques are used (Sponsor consulted), they will be documented in the final report.

Table of Statistical Comparisons

Control Group	Treatment Groups
1	2, 3, 4, 5

The above table defines the set(s) of comparisons to be used in the statistical analyses described below. If more than one set of comparisons is required, all analyses will be conducted separately on each set unless stated otherwise. Data for each sex within a set will also be analyzed separately.

The raw data will be tabulated within each time interval, and the mean and standard deviation will be presented for each endpoint by sex and group. For each endpoint, treatment groups will be compared to the control group using the analysis outlined below. Data for some endpoints, as indicated, will be transformed by either a log or rank transformation prior to conducting the specified analysis.

Endpoints	Type of Analysis
Body Weight Body Weight Gain Food Consumption Hematology (except Leukocyte Counts) Coagulation Clinical Chemistry Organ Weights Absolute Weights Relative to Body and Brain Weight	Group Pair-wise Comparisons
Leukocyte Counts Total Leukocyte Counts Differential Leukocyte Counts	Log Transformation Group Pair-wise Comparisons (Levene's/ANOVA-Dunnet's/Welch's)
Urinalysis Urine Volume Specific Gravity pH	Rank Transformation with Dunnett's Test
Mortality Data	Survival Analysis

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Tumor Data	Tumor Analysis
Non-Tumor Microscopic Pathology Data	To be determined if required

9.1. Group Pair-Wise Comparisons (Levene's/ANOVA-Dunnett's/Welch's)

If sample sizes for all groups are 3 or greater, Levene's test[4] will be used to assess homogeneity of group variances for each specified endpoint (see table above) and for all collection intervals. If Levene's test is not significant ($p \geq 0.01$), a pooled estimate of the variance (Mean Square Error or MSE) will be computed from a one-way analysis of variance (ANOVA) and utilized by a Dunnett's[5] comparison of each treatment group with the control group. If Levene's test is significant ($p < 0.01$), comparisons with the control group will be made using Welch's t-test[6] with a Bonferroni correction.

In the case that sample size is less than 3 for at least one treatment group, Levene's method cannot be implemented. Groups with sample sizes less than 3 will be excluded from the analysis and control-treatment pair-wise comparisons that satisfy the sample size assumption ($n \geq 3$) will be conducted using Welch's t-test with a Bonferroni correction.

If there are only 2 groups involved, the above methodology applies and the Dunnett's test reduces to a Student's t-test[7].

Results of all pair-wise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise.

9.2. Log Transformation with Group Pair-wise Comparisons

Historical data indicates that leukocyte counts (total and differential) are not normally distributed; therefore, a log transformation will be performed on these data. The transformed data will then be analyzed as described in the Group Pair-wise Comparisons section.

9.3. Rank Transformation with Dunnett's Test

Historical data indicate that this endpoint has unpredictable distribution characteristics, thus analysis would be enhanced by use of a non-parametric test. For each specified endpoint (see table above) and for each collection interval, a rank transformation will be performed. The transformed data will then be analyzed using Dunnett's test, to compare each treatment group with the control group.

If sample size for the control group is 2 or greater, Dunnett's test will be used to compare each treatment group having a non-zero sample size with the control group.

---

[4] Milliken GA, Johnson DE. Analysis of messy data. London: Chapman and Hall: 1992.
[5] Dunnett, CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 1955;50:1096-1121.
[6] Welch BL. The significance of difference between two means when the population variances are unequal. Biometrika 1937;29:350-362.
[7] Steel RGD, Torrie JH. Principles and Procedures of Statistics. A biometrical approach. New York: McGraw-Hill; 1980.

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If there are only 2 groups involved, the above methodology applies and the Dunnett's test reduces to a Student's t-test[7]. Results of all pair-wise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests unless indicated otherwise.

9.4. Survival Analysis

Intercurrent mortality data will be analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups will be conducted using a log-rank test[8]. If this overall test is significant ($p<0.05$) and there are more than two groups, then a follow up analysis will be done where each treatment group will be compared to the control group using a log-rank test.

Results of all pair-wise comparisons will be reported at the 0.05 and 0.01 significance levels. All endpoints will be analyzed using two-tailed tests.

9.5. Tumor Analysis

Tumor incidence data will be analyzed using both survival adjusted and unadjusted tests. The unadjusted tests will be based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test[9] will be calculated and Fisher's exact test[10] will be used to compare each treatment group with the control group. The survival adjusted test will be conducted according to the prevalence/mortality methods described by Peto et al.[11]. Evaluation criteria (p-values of significance) will be applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%)[12]. The evaluation criteria are given in the following table.

Evaluation Criteria for Common and Rare Tumors

Test for Positive Trends	Control-High Pair-wise Comparisons
Common and rare tumors will be tested at 0.005 and 0.025 significance levels, respectively	Common and rare tumors will be tested at 0.01 and 0.05 significance levels, respectively

Electronic data will be provided for this study with the final report. The format of the data sets will be prepared following the guidelines of the United States Environmental Protection Agency (EPA).

[8] Allison PD. Survival analysis using the SAS system: A Practical Guide. Cary (NC). SAS Institute Inc.; 1995.
[9] Agresti A. Categorical data analysis. 2nd ed. New York: John Wiley and Sons; 2002.
[10] Zar JH. Biostatistical Analysis. 4th ed. New Jersey: Prentice Hall; 1999.
[11] Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Pete J, Richards S, Wahrendorf J. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: Long-term and short-term screening assays for carcinogens: a critical appraisal. Annex to Supplement 2. p. 311-426. International Agency for Research on Cancer, Lyon; 1980.
[12] Haseman JK. A reexamination of false-positive rates for carcinogenesis studies. Fund Appl Toxicol 1983;3:334-339.

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10. STUDY REPORTS

10.1. Progress/Status Reports

Regular progress reports will be submitted to the Sponsor weekly for the first 5 weeks and biweekly reports through the first quarter (Week 13). Thereafter, progress reports will be sent approximately once per month.

10.2. Final Report

After completion of the study, a comprehensive draft report containing the results of all tests, analyses, observations and measurements required by this protocol, and an interpretative summary of the study results will be submitted to the Sponsor. The report will include all items required by the applicable regulatory agency. After receipt of any Sponsor comments, 1 copy (unbound) of the final report will be issued. An electronic copy (PDF) will be provided with the final report. This electronic copy will be searchable, hyperlinked (including headings, tables, figures, references, and all tables of contents), and bookmarked. One electronic copy will be in Microsoft Word, where possible. The electronic copies can be sent on CDs.

Six months after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor. Any modifications or changes to the draft report requested 6 months after issuance of the draft will be performed at additional cost to the Sponsor.

11. DATA AND SPECIMEN RETENTION

All raw data, documentation, records, protocol, specimens, samples and the final report generated as a result of this study will be retained at MPI Research or an MPI Research contracted archive facility for a period of 1 year following the issuance of the draft report. The Sponsor will be contacted annually by MPI Research Archive staff regarding the retained material and will be responsible for the incurred costs for the return, disposal, or continued storage of any study generated material retained after that time.

12. ANIMAL WELFARE

MPI Research is committed to complying with all applicable regulations governing the care and use of laboratory animals. Animal welfare for this study will be in compliance with the U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR Parts 1, 2 and 3). The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 1996, will be followed. This facility maintains an Animal Welfare Assurance statement with National Institutes of Health, Office of Laboratory Animal Welfare.

To ensure compliance:

A. This protocol will be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) before animal receipt or transfer.

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B. The Sponsor, by his or her approval, attests that the activities specified in this protocol do not unnecessarily duplicate any previous experiment.

C. The Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animal and has signified that (select one):

X i.) The relevant supervisory government agency currently gives no alternatives.

__ ii.) The following literature searches have been performed to determine whether an alternative species could be used or another procedure to reduce any pain or distress was available and none was found.

Date: November 17, 2009 Literature Search Reference Number: 0001
Interval Searched: All years to Present
Search terms: general alternative testing methods; alternative testing methods, toxicology; general toxicology testing method alternatives
Databases searched: toxnet.nlm.nih.gov;pubmed.gov;medscape.com;caat.jhsph.edu

__ iii.) This study does not require any procedures that may cause more than slight or momentary pain or distress to the animal. Note, unknown test articles are presumed to have the potential to cause more than slight pain or distress.

13. APPROVAL

13.1. Date of Sponsor Approval

_____
Date

13.2. Study Director Approval/Study Initiation

_____ _____
Lisa Craig, B.S. Date
Study Director

13.3. MPI Research Management Approval

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David G. Serota, Ph.D., D.A.B.T. Date
Senior Vice President, Drug Safety Evaluation

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Subject: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -
From: CN=Greg Schweer/OU=DC/O=USEPA/C=US
To: CN=Rose Allison/OU=DC/O=USEPA/C=US@EPA CN=Jim Alwood/OU=DC/O=USEPA/C=US@EPA
Cc:
Submit Time: 5/2/2010 16:59:00

Jim,

Did J. Hoover get in touch with you Friday and, if so, was anyone in RAD around to make a decision?

Greg Schweer
Chief, New Chemicals Management Branch
Chemical Control Division
U.S. EPA, Office of Pollution Prevention and Toxics
(202)564-8469

-----Forwarded by Greg Schweer/DC/USEPA/US on 05/02/2010 12:56PM -----

To: Greg Schweer/DC/USEPA/US@EPA
From: James R Hoover <James.R.Hoover@USA.dupont.com>
Date: 04/30/2010 03:57PM
Subject: Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Hi Greg....My personal apologies for this very late discovery, and even later communication to Rose and to you, on this issue. This is clearly our mistake, and I take full responsibility for it.

I called Rose to give her a heads-up on the enclosed EMails (below). I now understand from Rose's Voicemail that she is out today.

For us to proceed as indicated, I think we would need a "non-objection" Email from EPA relative to this EMail before 6:00am this coming Monday morning (May 3rd).

The details are show below.

Any advice would be much appreciated. We fully realize this may be difficult to impossible, but I wanted to make fully sure that what ever we do is totally right.

Again, my apologies.

Very best regards, Jim

Jim Hoover, FPS Global Regulatory Manager

DuPONT DCF/FPS
CRP 702, Room 2116
Wilmington, DE 19880

BBerry: Personal Phone / Ex. 6

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 03:40 PM -----

James R Hoover/AE/DuPont
04/30/2010 03:11 PM

To Allison.Rose@epamail.epa.gov

cc Gary W Jepson/AE/DuPont@DuPont, Steven R Frame/AE/DuPont@DuPont, Susan M Munley/AE/DuPont@DuPont, Jane Bradd Andersen/AE/DuPont@DuPont

Subject Fw: URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Hi Rose...Jane is away on vacation, and out of communication range.

My personal, and DuPont company, apologies for this late and urgent 'non-objection' request consideration, but our GenX Toxicity Team has just realized that we overlooked a CRITICAL study design issue in the 18405-1937 Mouse Study Blood Collection Amendment just approved by EPA.

The details are outlined in the Notes from Randy Frame and Sue Munley, shown below.

Given the circumstances and timing, what options do we have to proceed, with EPA agreement, for what Randy and Sue recommend (i.e. a non-objection to proceed).

We fully realize this may be difficult to impossible, but I wanted to make fully sure that what ever we do is totally right.

Many thx for your advice...best regards, Jim

Jim Hoover, FPS Global Regulatory Manager

DuPONT DCF/FPS
CRP 702, Room 2116
Wilmington, DE 19880

BBerry: **Personal Phone / Ex. 6**

----- Forwarded by James R Hoover/AE/DuPont on 04/30/2010 02:50 PM -----

Steven R Frame/AE/DuPont
04/30/2010 11:46 AM

To James R Hoover/AE/DuPont@DuPont, Jane Bradd Andersen/AE/DuPont@DuPont

cc Gary W Jepson/AE/DuPont@DuPont, Susan M Munley/AE/DuPont@DuPont

Subject URGENT ISSUE: 18405-1037 mouse study blood collection amendment -

Jim, Jane,

Please see Sue's note below. In order to get any useful results from the blood collection on adult females in the mouse study, we need to administer a dose on the day of sacrifice (in the current protocol, the last dose is the day before sacrifice). Without a day-of-sacrifice dosing, the blood data from the moms will be of little value. Therefore, it is near certain the EPA would concur with this minor change in procedure since they suggested the blood collection in the first place, and they undoubtedly want the most useful information. Further, this would have no effect on the study results since the animals are sacrificed very soon after the extra dose. Nevertheless, we will need your OK, and EPA's OK to proceed, and we must have this OK before Monday due to the stage of the test we are in. The new amendment could be to the EPA on Monday or soon thereafter.

Randy

----- Forwarded by Steven R Frame/AE/DuPont on 04/30/2010 11:38 AM -----

Susan M Munley/AE/DuPont
04/30/2010 11:37 AM

To Steven R Frame/AE/DuPont@DuPont, Gary W Jepson/AE/DuPont@DuPont

cc

Subject 18405-1037 mouse study blood collection amendment - URGENT ISSUE

While preparing to execute the blood collection for plasma TK as dictated by the recently-approved protocol amendment 4, we realized that we overlooked a CRITICAL study design issue.

As per protocol, adult animals are scheduled to be dosed through one day PRIOR to scheduled euthanasia.

Based on existing data, blood collection scheduled for two hours following the last dose is the optimal and most meaningful time for collection.

We cannot obtain enough volume for this work without making the bleed a terminal bleed.

Therefore, we need to write another amendment (draft attached below) to specify that animals will be administered a single additional dose on the morning of scheduled euthanasia and then euthanized two hours following that dose.

I am writing to seek non-objection to proceed with this beginning this coming Monday morning, May 3.

The first F0 females to reach PND 21 are scheduled to have their litters weaned and be subsequently euthanized this coming Monday morning.

As these procedures will clarify and improve upon the data dictated by the previously approved amendment 4, please let me know if we can proceed with approving this work to begin on Monday.

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189225 Draft Amendment 5 043010.doc 

From: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>
Subject: Repeat Dose Metabolism and Pharmacokenetics in Rats [OPPTS 870.7485]
To: Rose Allison/DC/USEPA/US@EPA
Submit Time: 6/3/2010 13:21:48

Dear Rose:

As a follow up to our conversation from Tuesday, June 1, 2010..... I am submitting for Agency approval modifications to the protocol for Repeat Dose Metabolism and Pharmacokenetics in Rats. This protocol was initially approved by the Agency on October 14, 2009. Subsequent to Agency approval DuPont has modified the protocol for the initiated study.

The following document is a copy of the protocol where the changes are embedded and highlighted using "track changes" tool for Microsoft Word. (See attached file: OPPTS 870.7485 Rat Track Changes)

The following is a copy of the protocol and changes as per the process employed by DuPont to satisfy GLP requirements.
(See attached file: DuPont-18405-1017 (rat)_final protocol.pdf)(See attached file: 18405-1017 (rat) Amendments to original protocol_wjf 19 may 2010.doc)

Kind regards,

Jane Bradd-Andersen
tel:302-999-2377
fax:302-999-2177
jane-bradd.andersen@usa.dupont.com

This communication is for use by the intended recipient and contains information that may be Privileged, confidential or copyrighted under applicable law. If you are not the intended recipient, you are hereby formally notified that any use, copying or distribution of this e-mail, in whole or in part, is strictly prohibited. Please notify the sender by return e-mail and delete this e-mail from your system. Unless explicitly and conspicuously designated as "E-Contract Intended", this e-mail does not constitute a contract offer, a contract amendment, or an acceptance of a contract offer. This e-mail does not constitute a consent to the use of sender's contact information for direct marketing purposes or for transfers of data to third parties.

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OPPTS 870.7485 Rat Track Changes  DuPont-18405-1017 (rat) final

protocol.pdf  18405-1017 (rat) Amendments to original protocol wjf 19 may 2010.doc 

The original draft protocol posted 21 July 2009 in a memo to Ms. Rose Allison was amended as noted below prior to final issue. The amendments are minor in nature and do not affect any of the objectives set forth in the original draft.

Protocol - 18405-1017 (rat)

1. Page 1, added Haskell Animal Welfare number BT196-P.

   Rationale: An approved animal use protocol number is required by Haskell's Animal Welfare Committee.

2. Page 5, Section 3, 1st paragraph, delete 2nd and 3rd sentence, delete the 2nd paragraph and replace with the following: When housed in metabolism units, feed will be supplied as ground chow.

   Rationale: Removed text regarding fasting and return of food, which was moved to section G, in-life; removed reference to chunk style chow and stainless steel feeders.

3. Page 6, section D, first bullet point, delete text regarding cannulated animals.

   Rationale: Cannulated animals will not be required for this study.

4. Page 6-7, section F, delete the last paragraph and replace with the following: LC/MS/MS will be used to confirm the chemical concentration of H-28548 in the dosing solution/suspension.

   Rationale: Identified the actual analytical method to be used for analysis.

5. Page 7, section G, delete the 2nd 1-line paragraph and insert the following text at the beginning: Rats will be housed individually in a metabolism unit and fasted for approximately 3 hours prior to dosing. Food will be returned approximately 2 hours post-dose.

   Rationale: Added information on housing, fasting and time to return food post-dose.

6. Page 7, section G, 2nd to last paragraph 2nd sentence change to: Cages will be rinsed with deionized water, which will be collected into a single container.

   Rationale: Use of deionized water is the preferred solvent for recovery of the test substance and analysis by LC/MS.

7. Page 7, section G, last paragraph, replace with the following: Per the testing guideline, if it is determined that a significant amount of the administered dose (<90%) is unaccounted for in the excreta (urine and feces, and cagewash, which is primarily dried excreta, then data on the percent of the total amount of H-28548 in collected tissues as well as residual carcass will be determined. Analysis of residual feed (if

collected) will only occur if a significant amount of the dose is still unaccounted for following the sequential and step-wise analysis of excreta (urine and feces), cagewash, tissues residual carcass, and residual feed (if collected). Total recovery should approximate 100 ± 10% of the amount of H-28548 administered.

Rationale: Clarified the strategy for determining a material balance of 100±10%; along with urine and feces analysis of cagewash was added to the primary samples tagged for analysis since pilot studies had suggested cagewash, which contains dried excreta (urine and feces), might be necessary for an acceptable material balance.

8. Page 8, section H, replace the 1st and 2nd sentences with the following: H-28548 will be quantitated in urine, feces and cagewash using LC/MS/MS, which may involve direct analysis (urine and cagewash) or solvent extraction (feces), followed by analysis. H-28548 will be quantitated in collected tissues, carcass, and residual feed (if collected) only if the mean recovery of the administered dose in urine and feces, and cagewash, which is primarily dried excreta, is <90%.

   Rationale: Added cagewash to the samples to be analyzed and specified use of LC/MS/MS as the analytical methodology.

9. Page 8, section I, added the following text to the end of the 2nd paragraph: Urine and fecal extracts may be filtered if necessary prior to LC/MS analysis.

   Rationale: Clarification; added analysis methodology.

10. Page 8, section I, 3rd paragraph, deleted the 1st sentence.

    Rationale: Clarification; 1st sentence was moved to previous paragraph.

11. Page 9, experimental start and termination dates, deleted "to be determined" and added actual start (March 2010) and termination dates (May 2010).

H-28548: Absorption, Distribution, Metabolism, and Elimination in the Rat

Work Request Number 18405

Service Code 1017

Protocol

Performing Laboratory: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

Haskell Animal Welfare
Committee Number: BT196-P

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INTRODUCTION

H-28548 is currently under investigation by the sponsor. The data from this study will provide basic information on the absorption, distribution, metabolism, and elimination (ADME) of H-28548 in the rat.

OBJECTIVE

The objective of this study is to determine the ADME of H-28548 in the rat following a single oral dose of H-28548 in water (Tier 1). Use of a non-radiolabeled test substance for determining a material balance and metabolite identification is justified based on results from an *in vitro* metabolism experiment with rat hepatocytes and rat oral and rat/monkey intravenous dose kinetic studies, which suggests that H-28548 is not metabolized and is eliminated rapidly.[1,2,3,4]

SPONSOR AND CONTACT INFORMATION

Sponsor:	E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.
Sponsor Contact:	Jane Bradd Andersen 302-999-2377 Jane-Bradd.Andersen@usa.dupont.com
Testing Facility Contact:	William J. Fasano, Sr. 302-451-3301 William.J.Fasano@usa.dupont.com
Sponsor Approval:	found on the Work Authorization Form

REGULATORY COMPLIANCE AND TEST GUIDELINES

This study will be conducted in compliance with the following good laboratory practice(s), which are compatible with current OECD Good Laboratory Practices:

- U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards

This study will be conducted in compliance with the following test guideline:

- U.S. EPA, OPPTS 870.7485. Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998)

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ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). The sponsor should make particular note of the following:

- The signature of the sponsor and/or the study director ensures that the study described in this protocol does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved will be painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee. The sponsor will be advised by the study director of all circumstances that could lead to this action in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- This protocol has been reviewed by the Haskell Animal Welfare Committee and complies with acceptable standards for animal welfare and humane care.
- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

MATERIALS AND METHODS

A. Test Substance

The test substance (CAS registry number 62037-80-3) will be supplied by the sponsor and assigned Haskell number 28548.

B. Test System

Male and female Crl:CD(SD) rats will be obtained from Charles River Laboratories, Inc. (Raleigh, North Carolina, U.S.A.).

The Sprague-Dawley rat was chosen for this study because of the extensive experience with this strain and its suitability with respect to longevity, sensitivity, and low incidence of spontaneous diseases. Furthermore, the Sprague-Dawley rat has been used previously for toxicokinetic and toxicity testing of this chemical.

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At the time of dosing, rats should be sexually mature, and the weight variation should not exceed ±20% of the mean weight by dose group. Each animal will be assigned a unique identification number to be used throughout the study. The last 3 digits of the animal identification number will be marked on the tail of each animal in indelible ink.

C. Animal Husbandry

1. Housing

During the pretest period, animals will be housed individually in solid bottom caging with bedding. Each cage rack may contain animals of either sex. Animals will be moved to metabolism units or lined solid bottom caging for the in-life phase of the study.

2. Environmental Conditions

Animal rooms will be maintained at a temperature of 18-26°C (64-79°F) and a relative humidity of 30-70%. Animal rooms will be artificially illuminated (fluorescent light) on an approximate 12 hour light/dark cycle. Unless judged by the study director or the laboratory veterinarian to have significantly affected the results of the study, the relative humidity and temperature ranges in the housing rooms will be recorded but will not be included in the final report.

3. Feed and Water

All animals will be provided tap water *ad libitum* and fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*. When housed in metabolism units, feed will be supplied as ground chow.

4. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

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D. Pretest Period

Upon arrival at DuPont Haskell, all rats will be housed in quarantine. The rats will be:

- quarantined for at least 6 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine and once prior to dosing.
- observed with respect to weight gain and any gross signs of disease or injury.

The animals will be released from quarantine by the laboratory animal veterinarian or designee based on body weights and clinical signs.

Animals that are accidentally killed or removed from study during the pretest period will be discarded without necropsy. Animals that are found dead or are sacrificed *in extremis* during the pretest period will be given a gross examination to check for the presence of disease. Dependant upon these findings, further diagnostic procedures may be employed at the discretion of the study director, a pathologist, or the laboratory animal veterinarian. The results will not be included in the final report unless considered significant to the evaluation of the study.

E. Assignment to Groups

Animals will be selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury. The weight variation of selected animals should not exceed ± 20% of the mean weight.

Each animal will be assigned an animal number and a cage identification number. The animal number and cage identification number will both be included on the cage label.

At study start, the animals should be at least 8 weeks old.

Animals that have not been assigned to a test group, or which have been removed from study prior to dosing for out-of-range body weights, will be released for other laboratory purposes or be sacrificed and discarded without anatomic pathology evaluation.

F. Dose Preparation, Analysis, and Rates

The test substance will be prepared for administration by oral gavage. This route was chosen because it is most commonly used for toxicity studies with H-28548.

H-28548 will be weighed into a vial and mixed with deionized water (dose vehicle). The dose solution will be prepared at a nominal concentration of 7.5 mg H-28548/mL (adjusted for purity), with a target dose level of 30 mg/kg body weight (bw) and a dose volume of 4 mL/kg bw. The dose level was chosen based on the results of the 28-day daily oral dosing study in rats, where the no-observed-adverse-effect level (NOAEL) was 30 and 300 mg/kg/day for males and females, respectively.[5]

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The dosing solution/suspension may be prepared on the day of use or prior to the day of use and stored refrigerated at 1-10°C.

LC/MS/MS will be used to confirm the chemical concentration of H-28548 in the dosing solution/suspension.

G. In-Life Phase - Tier 1

1. Material Balance and Tissue Distribution

Rats will be housed individually in a metabolism unit and fasted for approximately 16 hours prior to dosing. Food will be returned approximately 2 hours post-dose.

Five male and 5 female rats will be administered H-28548 at 30 mg/kg bw. Two male and 2 female rats will each be administered dose vehicle (deionized water at 4 mL/kg bw) for collection of control excreta and tissue samples. Rats will be returned to individual metabolism unit following dosing.

Urine and feces will be collected on dry ice predose and at 0-6, 6-12, 12-24, and every 24 hours until 168 hours post dose. Evidence supporting a lack of metabolism of H-28548 in rat hepatocytes and rat oral kinetic studies, precludes the necessity for a radiolabeled form of H-28548 and collection of expired air.

At the end of the experiment (168 hours post dose), rats will be killed by CO_2 asphyxiation followed by exsanguination. The following tissues (Tier 1) will be collected:

liver
fat
G.I. tract (and contents)
kidney
spleen
whole blood
residual carcass

After collection, these samples will be stored at approximately ≤-10°C.

Over the course of the experiment, residual feed will be collected (if necessary) into a single container and stored refrigerated at 1-10°C. Cages will be rinsed with deionized water, which will be collected into a single container. Cage wash will be stored at room temperature and/or refrigerated at 1-10°C.

Per the testing guideline, if it is determined that a significant amount of the administered dose (<90%) is unaccounted for in the excreta (urine and/or feces, and cagewash, which is primarily dried excreta) then data on the percent of the total amount of H-28548 in the collected tissues as well as residual carcass will be determined. Analysis of residual feed (if collected) will only occur if a significant amount of the dose is still unaccounted for following the sequential and step-wise analysis of excreta (urine and feces), cagewash, tissues, residual carcass, and residual

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feed (if collected). Total recovery should approximate 100 ± 10% of the amount of H-28548 administered.

H. Quantitation of H-28548

H-28548 will be quantitated in urine, feces and cagewash using LC/MS/MS, which may involve direct analysis (urine and cagewash) or solvent extraction (feces), followed by analysis. H-28548 will be quantitated in collected tissues, carcass, and residual feed (if collected) only if the mean recovery of the administered dose in urine and feces, and cagewash, which is primarily dried excreta, is <90%. Details of the methodologies employed will be documented in the study records and presented in the final report.

I. Quantification and Identification of Metabolites

The profile of H-28548 (and metabolites, if any) will be evaluated in urine and feces samples.

Samples may be pooled across animals for a given time interval. Feces samples will be extracted using appropriate solvent(s). Urine and fecal extracts may be filtered if necessary prior to LC/MS analysis.

It is expected that H-28548 will not be metabolized by the rat. However, any metabolites that are found to represent greater than 5% of the administered dose will be submitted for structural identification by mass spectroscopy or other suitable methods. The methodology relating to identification of H-28548 and metabolites will be documented in the study records and presented in the final report.

STATISTICAL ANALYSES

Group data will be represented as a mean ± SD. Other statistical evaluations may be performed at the discretion of the study director and sponsor.

CONTROL OF BIAS

Effective mixing of samples and avoidance of cross-contamination will be used to control bias during sample collection and analysis.

ADDITIONAL TESTING – TIER 2

Additional studies (Tier 2) may be added by amendment to this protocol if required. Studies at the Tier 2 level are designed to answer questions about the disposition of test chemicals based on the existing toxicology data base and/or results of Tier 1 testing. Only those studies that address a specific concern not resolved at Tier 1 will be conducted following a discussion with the Agency and a mutual agreed path forward.

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SAFETY AND HOUSEKEEPING

All chemicals used during this study will be handled according to the procedures specified in the MSDS and disposed of according to the Stine-Haskell Waste Disposal Guidelines and the area Safety, Health and Environmental (SHE) manual.

RECORDS AND SAMPLE STORAGE

All raw data, the protocol, amendments (if any), and the final report will be retained.

PROPOSED STUDY DATES

Experimental Start: March 2010

Experimental Termination: May 2010

REFERENCES

1. DuPont Haskell (2007). In Vitro Rat Hepatocyte Screen. Unpublished report, DuPont-23460.

2. DuPont Haskell (2008). Repeated Dose Oral Toxicity 7-Day Gavage Study in Rats. Unpublished report, DuPont-24009.

3. DuPont Haskell (2007). Biopersistence and Pharmacokinetic Screen in Rats. Unpublished report, DuPont-24281.

4. DuPont Haskell (2009). Cross-Species Comparison of FRD-902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing. Unpublished report, DuPont-17751-1579 RV1.

5. DuPont-Haskell (2008). A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery. Unpublished report, DuPont-24447.

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SIGNATURES

Approved by: _____ 16-MAR-2010

William J. Fasano, Sr., B.S.
Study Director

Date

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H-28548: Absorption, Distribution, Metabolism, and Elimination in the Rat

Work Request Number 18405

Service Code 1017

Protocol

Performing Laboratory: E.I. du Pont de Nemours and Company
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P.O. Box 50
Newark, Delaware 19714
U.S.A.

Haskell Animal Welfare
Committee Number: BT196-P

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INTRODUCTION

H-28548 is currently under investigation by the sponsor. The data from this study will provide basic information on the absorption, distribution, metabolism, and elimination (ADME) of H-28548 in the rat.

OBJECTIVE

The objective of this study is to determine the ADME of H-28548 in the rat following a single oral dose of H-28548 in water (Tier 1). Use of a non-radiolabeled test substance for determining a material balance and metabolite identification is justified based on results from an *in vitro* metabolism experiment with rat hepatocytes and rat oral and a rat/monkey intravenous dose kinetic studies, which suggests that H-28548 is not metabolized and is eliminated rapidly.[1,2,3,4]

SPONSOR AND CONTACT INFORMATION

Sponsor:	E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.
Sponsor Contact:	Jane Bradd Andersen 302-999-2377 Jane-Bradd.Andersen@usa.dupont.com
Testing Facility Contact:	William J. Fasano, Sr. 302-451-3301 William.J.Fasano@usa.dupont.com
Sponsor Approval:	found on the Work Authorization Form

REGULATORY COMPLIANCE AND TEST GUIDELINES

This study will be conducted in compliance with the following good laboratory practice(s), which are compatible with current OECD Good Laboratory Practices:

- U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards

This study will be conducted in compliance with the following test guideline:

- U.S. EPA, OPPTS 870.7485. Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998)

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ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). The sponsor should make particular note of the following:

- The signature of the sponsor and/or the study director ensures that the study described in this protocol does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved will be painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee. The sponsor will be advised by the study director of all circumstances that could lead to this action in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- This protocol has been reviewed by the Haskell Animal Welfare Committee and complies with acceptable standards for animal welfare and humane care.
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The Sprague-Dawley rat was chosen for this study because of the extensive experience with this strain and its suitability with respect to longevity, sensitivity, and low incidence of spontaneous diseases. Furthermore, the Sprague-Dawley rat has been used previously for toxicokinetic and toxicity testing of this chemical.

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At the time of dosing, rats should be sexually mature, and the weight variation should not exceed ±20% of the mean weight by dose group. Each animal will be assigned a unique identification number to be used throughout the study. The last 3 digits of the animal identification number will be marked on the tail of each animal in indelible ink.

C. Animal Husbandry

1. Housing

During the pretest period, animals will be housed individually in solid bottom caging with bedding. Each cage rack may contain animals of either sex. Animals will be moved to glass metabolism units or lined solid bottom caging for the in-life phase of the study.

2. Environmental Conditions

Animal rooms will be maintained at a temperature of 18-26°C (64-79°F) and a relative humidity of 30-70%. Animal rooms will be artificially illuminated (fluorescent light) on an approximate 12 hour light/dark cycle. Unless judged by the study director or the laboratory veterinarian to have significantly affected the results of the study, the relative humidity and temperature ranges in the housing rooms will be recorded but will not be included in the final report.

3. Feed and Water

All animals will be provided tap water *ad libitum* and fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*.

When housed in glass metabolism units, feed will be supplied as ground chow.

Deleted: Animals will be fasted before dosing with test substance. Food will be returned approximately 2 hours post-dose.

Deleted: chunk chow using a stainless steel feeder mounted on the inside rim of the glass metabolism unit

4. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

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The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Data are maintained separately from study records and may be included in the final report at the discretion of the study director.

D. Pretest Period

Upon arrival at DuPont Haskell, all rats will be housed in quarantine. The rats will be:

- quarantined for at least 6 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine and once prior to initiation of exposures.
- observed with respect to weight gain and any gross signs of disease or injury.

Deleted: with the exception of cannulated animals which will be quarantined for at least 3 days.

The animals will be released from quarantine by the laboratory animal veterinarian or designee based on body weights and clinical signs.

Animals that are accidentally killed or removed from study during the pretest period will be discarded without necropsy. Animals that are found dead or are sacrificed *in extremis* during the pretest period will be given a gross examination to check for the presence of disease. Dependant upon these findings, further diagnostic procedures may be employed at the discretion of the study director, a pathologist, or the laboratory animal veterinarian. The results will not be included in the final report unless considered significant to the evaluation of the study.

E. Assignment to Groups

Animals will be selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury. The weight variation of selected animals will not exceed ± 20% of the mean weight.

Each animal will be assigned an animal number and a cage identification number. The animal number and cage identification number will both be included on the cage label.

At study start, the animals will be at least 8 weeks old.

Animals that have not been assigned to a test group, or which have been removed from study prior to dosing for out-of-range body weights, will be released for other laboratory purposes or be sacrificed and discarded without anatomic pathology evaluation.

F. Dose Preparation, Analysis, and Rates

The test substance will be prepared for administration by oral gavage. This route was chosen because it is most commonly used for toxicity studies of H-28548.

H-28548 will be weighed into a vial and mixed with deionized water (dose vehicle). The dose solution will be prepared at a nominal concentration of 7.5 mg H-28548/mL (adjusted for purity), with a target dose level of 30 mg/kg body weight (bw) and a dose volume of 4 mL/kg bw. The

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dose level was chosen based on the results of the 28-day daily oral dosing study in rats, where the no-observed-adverse-effect level (NOAEL) was 30 and 300 mg/kg/day for males and females, respectively.[5]

The dosing solution/suspension may be prepared on the day of use or prior to the day of use and stored refrigerated at 1-10°C.

LC/MS/MS will be used to confirm the chemical concentration of H-28548 in the dosing solution/suspension.

Deleted: HPLC or another suitable analytical method will be used to confirm the chemical concentration of H-28548 in the dosing solution/suspension. The analytical method chosen will be documented in the study records and presented in the final report

G. In-Life Phase - Tier 1

1. Material Balance and Tissue Distribution

Rats will be housed individually in a metabolism unit and fasted for approximately 3 hours prior to dosing. Food will be returned approximately 2 hours post-dose.

Five male and 5 female rats will be administered H-28548 at 30 mg/kg bw. Two male and 2 female rat will each be administered dose vehicle (deionized water at 4 mL/kg bw) for collection of control excreta and tissue samples.

Urine and feces will be collected on dry ice predose and at 0-6, 6-12, 12-24, and every 24 hours until 168 hours post dose. Evidence supporting a lack of metabolism of H-28548 in rat hepatocytes and rat oral kinetic studies, precludes the necessity for a radiolabeled form of H-28548 and collection of expired air.

Deleted: Rats will be placed individually in an all-glass metabolism unit following dosing.¶

At the end of the experiment (168 hours post dose), rats will be killed by CO_2 asphyxiation followed by exsanguination. The following tissues (Tier 1) will be collected:

liver
fat
G.I. tract (and contents)
kidney
spleen
whole blood
residual carcass

After collection, these samples will be stored at approximately ≤-10°C.

Over the course of the experiment, residual feed will be collected into a single container and stored refrigerated at 1-10°C. Cages will be rinsed with deionized water, which will be collected into a single container. Cage wash will be stored at room temperature and/or refrigerated at 1-10°C.

Deleted: Cages will be rinsed with detergent and water (50:50 v/v), water, then acetone, and the combined rinse will be collected into a single container.

Per the testing guideline, if it is determined that a significant amount of the administered dose (<90%) is unaccounted for in the excreta (urine and feces, and cagewash, which is primarily dried excreta), then data on the percent of the total amount of H-28548 in collected tissues as well as residual carcass will be determined. Analysis of residual feed (if collected) will only

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occur if a significant amount of the dose is still unaccounted for following the sequential and step-wise analysis of excreta (urine and feces), cagewash, tissues and residual carcass, and residual feed (if collected). Total recovery should approximate 100 ± 10% of the amount of H-28548 administered.

H. Quantitation of H-28548

H-28548 will be quantitated in urine, feces and cagewash using using LC/MS/MS, which may involve direct analysis (urine and cagewash) or solvent extraction (feces), followed by analysis. H-28548 will be quantitated in collected tissues, carcass, residual feed (if collected), only if the mean recovery of the administered dose in urine and feces, and cagewash, which is primarily dried excreta, is <90%.

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Deleted: suitable methodology
Deleted: , tissues
Deleted: (HPLC-MS)
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Deleted: Details of the methodologies employed will be documented in the study records and presented in the final report.

I. Quantification and Identification of Metabolites

The profile of H-28548 (and metabolites, if any) will be evaluated in urine and feces samples.

Samples will be pooled across animals for a given time interval. Feces samples will be extracted using appropriate solvent(s). Urine and fecal extracts may be filtered if necessary prior to LC/MS analysis.

However, any metabolites that are found to be greater than 5% of the administered dose will be submitted for structural identification by mass spectroscopy or other suitable methods. The methodology relating to identification of H-28548 and metabolites will be documented in the study records and presented in the final report.

Deleted: Urine and fecal extracts may be filtered if necessary prior to HPLC analysis. It is expected that H-28548 will not be metabolized by the rat.

STATISTICAL ANALYSES

Group data will be represented as a mean ± SD. Other statistical evaluations may be performed at the discretion of the study director and sponsor.

CONTROL OF BIAS

Effective mixing of samples and avoidance of cross-contamination will be used to control bias during sample collection and analysis.

ADDITIONAL TESTING – TIER 2

Additional studies (Tier 2) may be added by amendment to this protocol if required. Studies at the Tier 2 level are designed to answer questions about the disposition of test chemicals based on the existing toxicology data base and/or results of Tier 1 testing. Only those studies that address a specific concern not resolved at Tier 1 will be conducted following a discussion with the Agency and a mutual agreed path forward.

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SAFETY AND HOUSEKEEPING

All chemicals used during this study will be handled according to the procedures specified in the MSDS and disposed of according to the Stine-Haskell Waste Disposal Guidelines and the area Safety, Health and Environmental (SHE) manual.

RECORDS AND SAMPLE STORAGE

All raw data, the protocol, amendments (if any), and the final report will be retained.

PROPOSED STUDY DATES

Experimental Start: March 2010 — Deleted: To be determined

Experimental Termination: May 2010 — Deleted: To be determined

REFERENCES

1. DuPont Haskell (2007). In Vitro Rat Hepatocyte Screen. Unpublished report, DuPont-23460.

2. DuPont Haskell (2008). Repeated Dose Oral Toxicity 7-Day Gavage Study in Rats. Unpublished report, DuPont-24009.

3. DuPont Haskell (2007). Biopersistence and Pharmacokinetic Screen in Rats. Unpublished report, DuPont-24281.

4. DuPont Haskell (2009). Cross-Species Comparison of FRD-902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing. Unpublished report, DuPont-17751-1579 RV1.

5. DuPont-Haskell (2008). A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery. Unpublished report, DuPont-24447.

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SIGNATURES

Approved by: _____ _____
William J. Fasano, Sr., B.S. Date
Study Director

ED_002003A_00068269-00010

Submit Time: 10/15/2009 20:29:56
From: CN=Rose Allison/OU=DC/O=USEPA/C=US
To: "Patel, Yogesh P" <Yogesh.P.Patel@wv.gov>
Cc:
Subject: RE: Request for conference call

Yogesh, Thanks for the list of questions. I am working with EPA attorneys about what I need from the Company as far as a confidentiality waiver so that we can have the conference call. US EPA does not have any overall agreement about sharing confidential information with the states. Perhaps we could arrange a time to talk so that we know approximately when you and your colleagues would be available for a call. I'm out tomorrow but expect to be in on Monday. Rose

---

Rose Allison
202/564-8970/FAX 202/564-9490

"Patel, Yogesh P" ---10/13/2009 03:19:38 PM---Rose, Following type of question we would like to ask you if possible:

From: "Patel, Yogesh P" <Yogesh.P.Patel@wv.gov>

To: Rose Allison/DC/USEPA/US@EPA

Date: 10/13/2009 03:19 PM

Subject: RE: Request for conference call

Rose,

Following type of question we would like to ask you if possible:

1. First of all what is this chemically (GenX)?
2. Can it react with water? What is the reaction ? What kind of end product may form from the reaction?
3. Is it harmful to aquatic life? What kind of study has been done to prove the answer!
4. Is it harmful to human life? What kind of supporting document we have to back it up our answer!
5. Is dilution is a solution for this compound? Is any treatment system available to treat this? If answer is yes, can it cause any problem when we put this stuff in the regular landfill or we need to dispose off on hazardous waste manner!
6. Can it travel in the river with the flow? Do we need to worry about closet water intake.
7. Can it go away with time or it can be accumulated in the river may cause problem in future!

Let me know your availability so I can arrange a conference call.

Thanks
Yogesh

-----Original Message-----
From: Allison.Rose@epamail.epa.gov [mailto:Allison.Rose@epamail.epa.gov]

Sent: Wednesday, October 07, 2009 3:13 PM
To: Patel, Yogesh P
Subject: Request for conference call

Hi Yogesh Patel, As I said on my phone message, I need to know the premanufacture notice (PMN) number/numbers that you want to talk about. Also so that I can get the appropriate people on the conference call I'll need to discuss with you what you want to talk about,-- something like a proposed agenda, and who would be on the conference call from WV and their backgrounds or titles. This conference call (1 hour) could possibly happen next week Wed. or Thurs. depending on people's availability, if we work out any Confidential Business Information (CBI) issues. I cannot use CBI in the email, so any reply should be by phone or information that is not claimed confidential. Regards, Rose Allison
PS I could be available to talk tomorrow at 10:15 am or after 3:00 pm or Friday preferably in the morning 10:15 am or 11:00 am.

Rose Allison
Deliveries
Senior Specialist
New Chemicals Program
Chemical Control Division (7405M)
US EPA
20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

For
EPA East Building
*1201 Constitution Ave NW
Room 4419H
**Wash DC

Submit Time: 6/18/2010 19:39:47
From: CN=Rose Allison/OU=DC/O=USEPA/C=US
To: CN=Jennifer Seed/OU=DC/O=USEPA/C=US@EPA
Cc:
Subject: Fw: Modified 1-generation Reproduction Study [OPPTS 870.3550]

Jennifer, These are the protocol modifications in this study to add the plasma collections. The track changes tool highlights the changes in red. With quick perusal they look to be additions for the females. Please scroll to the end for attachments. Can I assume that they're ok? Rose

Rose Allison For Deliveries
Team Leader **EPA East Building**
New Chemicals Program *1201 Constitution Ave NW *
Chemical Control Division (7405M) **Room 4419G**
US EPA **Wash DC 20004**
1200 Pennsylvania Ave. NW
Washington, DC 20460
202/564-8970/FAX 202/564-9490

----- Forwarded by Rose Allison/DC/USEPA/US on 06/18/2010 03:33 PM -----

From: Jane Bradd Andersen <JANE-BRADD.ANDERSEN@usa.dupont.com>

To: Rose Allison/DC/USEPA/US@EPA

Date: 06/08/2010 10:30 AM

Subject: Modified 1-generation Reproduction Study [OPPTS 870.3550]

Dear Rose:

As a follow up to our conversation from Tuesday, June 1, 2010..... I am submitting for Agency approval modifications to the protocol for Modified One-Generation Reproduction Study in Mice. This protocol was initially approved by the Agency on November 2009. The Agency provided approval for Amendment 4 on April 28, 2010 and Amendment 5 on May 3, 2010 [see attached emails]. DuPont recognized other changes have occurred to the protocol subsequent to the initial Agency approval.

With this email I am requesting Agency approval for Amendments 1 through 3 to the protocol for Modified One-Generation Reproduction Study in Mice.

The following document is a copy of the protocol where the changes are embedded and highlighted using "track changes" tool for Microsoft Word.

The following is a copy of the protocol and changes as per the process employed by DuPont to satisfy GLP requirements.

Kind regards,

Jane Bradd-Andersen
tel:302-999-2377
fax:302-999-2177
jane-bradd.andersen@usa.dupont.com

an

http://www.DuPont.com/corp/email_disclaimer.html

189225 Draft Amendment 5 043010.doc 189225 Draft Amendment 5 043010.doc 18405-1037 complete

protocol smm 19 may 2010.pdf 18405-1037 complete protocol smm 19 may 2010.pdf 18405-1037 protocol with amendments as tracked changes smm june 3,

2010.pdf 18405-1037 protocol with amendments as tracked changes smm june 3, 2010.pdf

PROTOCOL

AN ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY OF H-28548 IN MICE

(U.S. EPA OPPTS 870.3550 and OECD Guideline 421)

Submitted To:

E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898

DuPont Work Request Number: 18405
DuPont Service Code: 1037
DuPont Study Number: 18405-1037

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

To provide preliminary information on the potential adverse effects of the test substance on male and female reproduction within the scope of a screening study. This will encompass gonadal function, mating behavior, conception, parturition and lactation of the F_0 generation and the development of offspring from conception through day 40 of postnatal life.

This study is subject to the applicable regulations of the Organisation for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Guideline 421, Reproduction/Development Toxicity Screening Test, July 27, 1995, and the United States Environmental Protection Agency (EPA) Health Effects Test Guidelines OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test, July 2000 and will be conducted in accordance with the EPA/TSCA and FIFRA (40 CFR Part 792 and 40 CFR Part 160) and the OECD Principles of Good Laboratory Practice.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Study Representative:

Susan M. Munley, MA
Research Toxicologist
Developmental, Reproductive and Neurobehavioral Toxicology
DuPont Haskell Laboratory for Health and Environmental Sciences
1090 Elkton Rd., PO Box 50
Newark, DE 19714
Tel: (302) 366-5240
Email: susan.m.munley@usa.dupont.com

2.2 Principal Investigator, Pathology

Greg P. Sykes, VMD, DACVP, DACLAM, DABT
PharmPath, LLC.
105 Phillips Mill Rd.
West Grove, PA, 19390-9165
Tel: (302) 451-3551
Cellular Tel: (484) 678-4433
Email: greg.p.sykes@usa.dupont.com

2.3 WIL Study Director:

Tammye L. Edwards, BS, LAT
Staff Toxicologist, Developmental and Reproductive Toxicology
WIL Research Laboratories, LLC
1407 George Road
Ashland, Ohio 44805
Tel: (419) 289-8700 ext. 2105
Fax: (419) 289-3650
Email: tledwards@wilresearch.com

2.4 WIL Departmental Responsibilities:

Eddie D. Sloter, PhD
Senior Toxicologist, Developmental
and Reproductive Toxicology
Emergency Contact
Tel: (419) 289-8700
Fax: (419) 289-3650
Email: esloter@wilresearch.com

Mark D. Nemec, BS, DABT
President and Chief Operating Officer

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

George A. Parker, DVM, PhD, DACVP, DABT
Director, Pathology

Melissa J. Beck, PhD
Assistant Director, Neurosciences

Daniel W. Sved, PhD
Director, Metabolism and Analytical Chemistry

Walter R. Miller, BS, DVM
Clinical Veterinarian,
Head of Surgery and Experimental Medicine

Ronald E. Wilson, BS
Director, Informational Systems

Carol A. Kopp, BS, LAT
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

Bennett J. Varsho, MPH, DABT
Operations Manager, Developmental and
Reproductive Toxicology and the Formulations Laboratory

Robert A. Wally, BS, RAC
Manager, Reporting and Regulatory
Technical Services

3 STUDY SCHEDULE:

Proposed Experimental Starting (Animal Receipt) Date:	5 January 2010
Proposed Experimental Start (First Day of Dosing) Date:	14 January 2010
Proposed Experimental Completion/Termination Date:	4 June 2010
Proposed Audited Report Date:	To be determined

4 TEST SUBSTANCE DATA:

4.1 Test Substance Shipment:

Test substance and applicable documentation, including a Certificate of Analysis, will be shipped under Sponsor's responsibility to:

Formulations Laboratory (WIL-189225; Tammye Edwards)
Attn: Larry Blessing
WIL Research Laboratories, LLC
1407 George Road
Ashland, Ohio 44805-8946

4.2 Identification:

H-28548 or HFPO Dimer Acid Ammonium Salt

4.3 **Haskell Test Substance Number:**

H-28548

4.4 **Lot Number:**

E109540-44A

4.5 **Expiration/Retest Date:**

13 June 2011

4.6 **Purity:**

84%

4.7 **Storage Conditions:**

Controlled room temperature and humidity (approximately 18° to 24°C and 20% to 70% relative humidity)

4.8 **Stability:**

The analysis was performed by the Sponsor and documented on the Certificate of Analysis.

4.9 **Physical Description:**

To be documented by WIL Research Laboratories, LLC.

4.10 **Reserve Samples:**

Reserve samples of the test substance will be taken in accordance with WIL Standard Operating Procedures and stored in the Archives at WIL Research Laboratories, LLC indefinitely, unless otherwise specified.

4.11 **Personnel Safety Data:**

See the Material Safety Data Sheet (MSDS) provided by the Sponsor.

4.12 **Test Substance Disposition:**

With the exception of the reserve sample for each batch of test substance, which will be archived as described, all neat test substance remaining at completion of the in-life phase of the study will be kept for subsequent studies.

5 TEST SYSTEM:

5.1 Species:

Mouse

5.2 Strain:

Charles River Crl:CD1(ICR)

5.3 Source:

Males: Charles River Laboratories, Inc., Raleigh, NC
Females: Charles River Laboratories, Inc., Kingston, NY

5.4 Number on Study:

100 males and 100 females (minimum of 120 males and 120 females purchased; males and females will be ordered from separate facilities to ensure the avoidance of sibling mating). Animals not assigned to study will be transferred to the stock animal colony or will be euthanized by carbon dioxide inhalation and the carcasses discarded.

The number of animals used on this study is consistent with OPPTS and OECD guidelines for reproduction/developmental toxicity screening studies.

5.5 Body Weight Range:

A minimum of 20 grams at randomization.

5.6 Approximate Age:

42-63 days old at randomization.

5.7 Identification System:

Each mouse will be uniquely identified by tattoo markings applied to the tail. Individual cage cards will be affixed to each cage and will display the animal number, group number, study number, dosage level and sex of the animal.

5.8 Justification for Selection:

This species and strain of animal is recognized as appropriate for reproduction studies. WIL Research Laboratories, LLC has reproductive historical control data in the Crl:CD1(ICR) mouse. This animal model has been proven to be susceptible to the effects of reproductive toxicants.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

The animals will be housed, 2-3 per cage, for at least 3 days following receipt. Thereafter, the mice will be housed individually. The F_0 males and females will be individually housed in solid bottom cages (plastic maternity cages) containing ground corncob nesting material (Bed-O' Cobs®) in an environmentally controlled room during the quarantine period and throughout the entire study until euthanasia. All F_1 offspring not euthanized at weaning will be housed by litter in the plastic cages with nesting material until postnatal day (PND) 28. F_1 offspring not selected for the maturation phase will be necropsied on PND 21. On PND 28, F_1 offspring will be individually housed in solid bottom cages (plastic maternity cages) containing ground corncob nesting material (Bed-O' Cobs®). The cages will be subject to routine cleaning at a frequency consistent with maintaining good animal health and WIL Standard Operating Procedures. The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain temperature at 71 ± 5°F (22 ± 3°C) and relative humidity at 50 ± 20%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting controlled by light timers will provide illumination for a 12-hour light/dark photoperiod. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Reverse osmosis-purified water will be available *ad libitum*. Filters servicing the automatic watering system are changed regularly according to WIL Standard Operating Procedures. The municipal water supplying the laboratory is analyzed according to WIL Standard Operating Procedures on a routine basis to ensure that contaminants are not present in concentrations that would be expected to affect the outcome of the study.

6.4 Basal Diet:

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 will be offered *ad libitum* during the study. Periodic analyses of the certified feed are performed by the manufacturer to ensure that heavy metals and pesticides are not present at concentrations that would be expected to affect the outcome of the study. Results of the analyses are provided to WIL Research Laboratories,

LLC by the manufacturer. Feeders will be changed and sanitized once per week.

6.5 Enrichment:

All animals will be offered Nestlets™ for enrichment that will be replaced as needed.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Quarantine:

Each animal will be inspected by a qualified technician upon receipt. Mice judged to be in good health and suitable as test animals will be immediately placed in quarantine for a minimum of 9 days. All mice will be initially weighed, permanently identified by tattoo markings applied to the tail and receive a clinical observation. During the quarantine period, each mouse will be observed twice daily for changes in general appearance and behavior. Prior to the start of the in-life phase, those animals judged to be suitable test subjects will be identified and receive a detailed physical examination.

7.2 Randomization:

At the conclusion of the quarantine period, animals judged to be suitable test subjects and meeting acceptable body weight requirements, will be assigned at random using a computer program. At that time, the animal numbers and corresponding body weights will be entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers and individual group assignments will be generated based on body weight stratification into a block design. Animals will then be arranged into the groups according to the printout. The control group and three test item groups will consist of 20 males and 20 females each.

Any animal assigned to the study that is found dead, euthanized *in extremis* or exhibits abnormal clinical signs, reduced food consumption or body weight losses prior to the start of dosing may be replaced by an animal of appropriate age when possible. Replacement animals will be arbitrarily assigned (not computer randomized) to the study based on comparable body weights (if possible) with respect to the animal that was replaced.

7.3 Route and Rationale of Test Item Administration:

The route of administration will be oral (gavage). Historically, this route has been used extensively for studies of this nature. Appropriately sized flexible,

Teflon®-shafted, stainless steel ball-tipped dosing cannulae will be used for the oral administration by gavage.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The dose levels proposed for the current study are 0, 0.1, 0.5, and 5 mg/kg/day and are based on previous and ongoing general toxicity studies in mice. These levels are currently being tested in an ongoing (in-life dosing phase complete) subchronic toxicity 90-day gavage study (DuPont-18405-1307). The doses for the 90-day gavage study were based on results from a previous 28-day gavage study (DuPont-24459) in which doses of 0, 0.1, 3, and 30 mg/kg/day were tested.

The following table presents the study group arrangement.

Group Number	Test Item	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Male	Female
1	Vehicle Control[b]	0	0	10	25	25
2	H-28548	0.1	0.01	10	25	25
3	H-28548	0.5	0.05	10	25	25
4	H-28548	5	0.5	10	25	25

[a] Dosage levels will be corrected for the purity of 84%.
[b] Deionized Water

7.4.2 Vehicle Control Item:

Deionized Water

7.4.3 F_0 Treatment Regimen:

The test and control items will be administered once daily at approximately the same time each day as follows:

7.4.3.1 Males:

F_0 males will be dosed for a minimum of 70 days prior to mating and continuing until the day prior to the scheduled euthanasia.

7.4.3.2 Females:

F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 21 for females that deliver. For females

that do not have positive signs of mating or delivery, dosing will continue until one day prior to euthanasia.

7.4.3.3 F_1 Males and Females:

F_1 males and females will be dosed beginning in PND 21 until one day prior to euthanasia.

7.4.4 Adjustment of Dosages:

Individual dosages will be calculated based on the most recent body weight to provide the proper mg/kg/day dosage.

7.5 Preparation and Analysis of Test Item Formulations:

7.5.1 Method and Frequency of Preparation:

Based on the physical characteristics of the test substance, appropriate methods will be used to ensure the best possible formulations of the test substance in the vehicle. Dosing formulations will be stored refrigerated (2-8°C) for a maximum of 12 days. The Study Director or designee will visually inspect the formulations prior to the initiation of dosing. This visual inspection will be performed to ensure that the formulations are visibly homogeneous and acceptable for dosing. Any special procedures required for formulation will be documented according to Good Laboratory Practices and presented in the final report of this study. Test substance formulations will be prepared approximately weekly and divided into aliquots for daily dispensation. The test substance and vehicle formulations will be stirred continuously during dosing.

7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Substance Formulations:

Stability and resuspension homogeneity were established on a previous study (Haas, Draft; WIL-189216). Test substance formulations were stable and 12 days of room temperature storage or refrigerated storage (2-8°C) at concentrations of 0.01 mg/mL and 100 mg/mL and homogenous following resuspension after 12 days of refrigerated storage (2-8°C). Stability and resuspension homogeneity will not be conducted on this study.

Homogeneity and concentration will be conducted on the first formulations prepared for dosing. Four 1-mL samples will be collected from the top, middle and bottom of the test substance formulations from the low and high dose groups and the samples analyzed to assess the

homogeneity of the test substance in the mixtures; the middle strata will serve as the measure of test substance concentration. Four 1-mL samples will be taken from the middle on the control and the mid-dose groups and analyzed for concentration of the test substance.

Concentration will be assessed on Week 4, 8, 12, 16 and 19 formulations prepared for dosing. Four 1-mL samples will be collected from the middle of each test substance formulation and the control group and analyzed for test substance content.

7.5.3 Sample Analysis:

Samples will be transferred to the Analytical Chemistry Department at WIL Research Laboratories, LLC for analysis. Analyses of test article formulations will be performed using a method developed and validated by WIL Research Laboratories, LLC. Initially, two of each set of four replicate, 1-mL samples will be analyzed; the remaining two 1-mL samples will be stored frozen (approximately -20°C) at WIL and will function as back-up samples. Back-up samples will be analyzed if requested by the Sponsor or Study Director or may be discarded following results that are within specifications and approval of the Study Director.

7.6 F_0 Breeding:

After a minimum of 70 days for males and 14 days of exposure for females, of exposure, one female will be cohabitated with one male mouse of the same treatment group, avoiding sibling mating, in a plastic cage for mating. Detection of mating will be confirmed by evidence of sperm in the vaginal lavage. After confirmation of mating, the female will be returned to an individual plastic cage and the day will be designated as day 0 of gestation.

A maximum of 14 days will be allowed for mating. After 14 days of mating, any females who have not shown evidence of breeding will be placed in a plastic cage containing nesting material.

7.7 F_0 Parturition and Lactation and F_1 Litters:

The day parturition is initiated will be designated as day 0 of lactation. Any difficulties at the time of parturition will be recorded. When parturition is judged to be complete, the sex of each pup will be determined, pups will be examined for gross malformations and the number of stillbirths and live pups will be recorded. Any changes or abnormalities in nesting and nursing behavior will be recorded. The dam and litter will remain together until postnatal day (PND) 21.

7.8 Identification of F_1 Litters:

Upon completion of delivery, all pups will be individually identified by tattoo markings applied to the digits. To reduce variability among the litters, on PND 4, eight pups of equal sex distribution (if possible) from each litter will be randomly selected. For litters consisting of fewer than eight pups, adjustments for litter sizes will not be performed. Following selection, the non-selected PND 4 pups will be euthanized by an intraperitoneal injection of sodium pentobarbital and discarded.

7.9 General Observations During the Experimental Period:

7.9.1 Parental Appearance and Behavior:

Each parental mouse (F_0) will be observed twice daily for moribundity and mortality, once in the morning and once in the afternoon. A detailed physical examination will be conducted weekly. Mortality and all signs of overt toxicity will be recorded on the day observed. The observations shall include, but are not limited to, evaluations for changes in appearance of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior. During the period of expected parturition, the dams will be observed twice daily for dystocia, prolonged labor, delayed labor or other difficulties at parturition. All animals will also be observed on the day of necropsy and findings will be recorded.

During the treatment period, each animal will be observed at approximately 1-2 hours following each dose administration for findings that are potentially related to treatment of that might change before the next scheduled observation. Additional post dosing observation periods may be necessary and will be documented in the study records.

7.9.2 Parental Body Weights:

All animals will have a final body weight recorded on the day of euthanasia.

7.9.2.1 Males:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until euthanasia.

7.9.2.2 Females:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until evidence of copulation is observed and on gestation days 0, 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.

For females with no evidence of mating, individual body weights will continue to be recorded on a weekly basis until euthanasia.

7.9.3 Parental Food Consumption*:

Individual food consumption will not be recorded during the breeding period because the animals are cohabitated at that time.

7.9.3.1 Males:

Recorded individually on a weekly basis, beginning on the first day of dose administration, until euthanasia.

7.9.3.2 Females:

Recorded individually on a weekly basis beginning on the first day of dose administration, until the start of the mating period. Individual food consumption will be recorded on the day evidence of copulation is observed (GD 0) and on gestation days 4, 7, 11, 14, 17 and 20 and lactation days 1, 4, 7, 14 and 21.

For females with no evidence of mating, individual food consumption will continue to be recorded on a weekly basis following the end of the mating period until euthanasia.

7.9.4 Examination of Offspring:

7.9.4.1 Appearance and Behavior:

All pups will be observed daily for general appearance and behavior and survival during lactation. A detailed physical examination will be recorded for each pup on PND 1, 4, 7, 14 and 21. Any abnormalities in nesting and nursing behavior will be recorded. The pups will be sexed on PND 0, 4, 14 and 21.

7.9.4.2 Body Weights:

Each pup will be weighed on PND 1, 4, 7, 14 and 21.

7.9.5 Pup Deaths:

7.9.5.1 Pups 0 to 4 Days of Age:

Moribund pups will be euthanized by an intraperitoneal injection of sodium pentobarbital. Stillborn pups, pups found dead between birth and PND 4, and any pups that are euthanized *in extremis* will be dissected (including the heart and the brain examined by a mid-coronal slice) by a technique described by Stuckhardt and Poppe (Stuckhardt and Poppe, 1984). If a skeletal anomaly is suspected, the pups will be eviscerated, cleared and stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined. Representative specimens with malformations may be preserved in 10% neutral buffered formalin at the discretion of the study director.

7.9.5.2 Pups 5 Days of Age to Weaning:

Moribund pups will be euthanized by an intraperitoneal injection of sodium pentobarbital (prior to PND 11) or by carbon dioxide inhalation. A gross necropsy will be performed on pups found dead or euthanized *in extremis*, and gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin. If a skeletal anomaly is suspected, the pups will be eviscerated, cleared and stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined.

7.10 Selection of F_1 Generation and Termination of PND 21 Nonselected Pups:

One male and one female pup per litter will be selected for the F_1 generation on or prior to PND 21. Only pups not expected to survive due to notable physical limitations will not be available for selection. A detailed evaluation of each pup excluded from selection will be recorded.

All PND 21 pups not selected for the F_1 generation will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

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7.11 Euthanasia of F_0 Generation:

7.11.1 Females:

7.11.1.1 Females Which Deliver:

On lactation day 21, all F_0 females that delivered will be euthanized by carbon dioxide inhalation. A gross examination will be performed and tissues preserved as described in Section 8.1. The number of former implantation sites will be recorded. Organ weights will be collected and tissues preserved as described in Section 8.2.

7.11.1.2 Females Which Fail to Deliver:

On post-mating day 25 (females with evidence of copulation) or post-cohabitation day 25 (females without evidence of copulation), the F_0 females which fail to deliver will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed and tissues will be preserved as described in Section 8.1. Organ weights will be collected as described in Section 8.2 with the exception of any ammonium sulfide stained uterus, which will be discarded. Uteri which appear nongravid by macroscopic examination will be opened and placed in a 10% ammonium sulfide solution (Salewski, 1964) for detection of early implantation loss.

7.11.1.3 Females with Total Litter Loss:

Females with total litter loss will be euthanized by carbon dioxide inhalation on the same day. The number of former implantation sites will be recorded and the number of corpora lutea (if litter loss occurs on or before PND 4) will be recorded. A gross necropsy examination will be performed and tissues preserved as described in Section 8.1. Organ weights will be collected as described in Section 8.2.

7.11.1.4 F_0 Deaths and Animals Euthanized *in Extremis*:

Females not surviving until the scheduled euthanasia will have a gross necropsy examination performed and tissues preserved as described in Section 8.1. Animals not expected to survive to the next observation period (moribund) will be euthanized by carbon dioxide inhalation and have a gross necropsy examination performed and tissues preserved as described in Section 8.1.

Organ weights will not be collected from found dead or euthanized *in extremis* females. The number and location of implantation sites or scars will be recorded for females dying or euthanized during gestation and lactation. The number of corpora lutea will be recorded for females dying or euthanized during gestation and up to and including lactation day 4. Uteri which appear nongravid by macroscopic examination will be opened and placed in a 10% ammonium sulfide solution (Salewski, 1964) for detection of early implantation loss.

Viable fetuses will be euthanized by an intrathoracic injection of sodium pentobarbital. Recognizable fetuses will be examined externally for gross abnormalities. Representative specimens with malformations may be preserved in 10% neutral-buffered formalin, at the discretion of the study director. For females found dead or euthanized *in extremis* during lactation, all pups will be examined externally and subjected to a necropsy examination according to Section 7.9.5.

7.11.2 Males:

Following completion of the mating period, all F_0 males will be euthanized by carbon dioxide inhalation and subjected to a gross necropsy and tissue preservation as described in Section 8.1. Organ weights will be collected as described in Section 8.2.

Males not surviving until the scheduled euthanasia will be subjected to a gross necropsy and tissue preservation as described in Section 8.1. Any males not expected to survive to the next observation period (moribund) will be euthanized by carbon dioxide inhalation and also necropsied and have tissues preserved as described in Section 8.1. Organ weights will not be collected.

7.12 F_1 Generation General Observations During The Experimental Period:

7.12.1 F_1 Clinical Observations:

Following weaning and selection, the mice will be observed twice daily for moribundity and mortality, once in the morning and once in the afternoon. Clinical observations will be recorded daily. Mortality and all signs of overt toxicity will be recorded on the day observed. The observations shall include, but are not limited to, evaluation for changes in appearance of the skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system function,

somatomotor activity and behavior patterns. All animals will also be observed on the day of necropsy and any findings will be recorded.

During the treatment period, each animal will be observed at approximately 1-2 hours following each dose administration for findings that are potentially related to treatment of that might change before the next scheduled observation. Additional post dosing observation periods may be necessary and will be documented in the study records.

7.12.2 F_1 Body Weights and Food Consumption:

F_1 males and females will be have a body weight recorded approximately weekly, beginning with the start of test diet administration until euthanasia (PND 21, 28, 35 and 40). All animals will have a final body weight recorded on the day of euthanasia.

F_1 males and females will have food consumption recorded individually on an approximately weekly basis beginning on PND 28 until euthanasia (PND 28, 35 and 40). Food consumption will not be collected from PND 21 to PND 28 during group housing for the F_1 males and females.

7.13 F_1 Postweaning Developmental Landmarks:

Offspring selected for the F_1 generation will be evaluated for attainment of the following landmarks of sexual maturity:

7.13.1 Balanopreputial Separation:

Each male pup will be observed for balanopreputial separation beginning on PND 25 as described by Korenbrot *et al.* (Korenbrot 1977). Examination of the males will continue daily until balanopreputial separation is present. The body weight of each male will be recorded on the day of attainment of balanopreputial separation.

7.13.2 Vaginal Patency:

Each female pup will be observed for vaginal patency beginning on PND 21 (only those selected for the F_1 generation) as described by Adams *et al.* (Adams 1985). Examination of the females will continue daily until vaginal patency is present. The body weight of each female will be recorded on the day of attainment of vaginal patency.

7.14 Euthanasia of F_1 Generation:

7.14.1 Scheduled Necropsy

On PND 40, all F_1 animals will be euthanized by carbon dioxide inhalation. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

7.14.2 Unscheduled Deaths or Animals Euthanized *in Extremis*

Any F_1 animals not surviving until the scheduled euthanasia or not expected to survive to the next observation period (euthanized by carbon dioxide inhalation) will be necropsied. A gross necropsy examination will be performed with an emphasis on evaluation of developmental morphology and organs of the reproductive system. Any gross lesions will be saved for possible future histopathological examination in 10% neutral buffered formalin.

8 ANATOMIC PATHOLOGY:

8.1 Macroscopic Examination:

A complete necropsy will be conducted on all F_0 parental animals dying spontaneously, euthanized *in extremis* (by carbon dioxide inhalation) or at termination. This will include examination of the external surface, all orifices, the cranial cavity, the external surface of the brain and the thoracic, abdominal and pelvic cavities including viscera. For F_0 females, the number of former implantation sites will be recorded.

At the time of necropsy, the following tissues and organs will be collected and placed in 10% neutral-buffered formalin (except as noted):

Coagulating gland	Prostate
Kidneys (2)	Seminal vesicles (2)
Liver	Testes with epididymides (2)[a]
Mammary gland (females only)	and vas deferens
Ovaries and oviduct (2)	Uterus[b] with cervix and vagina
Pituitary	All gross lesions[c]

a - Testes and epididymides will be fixed in Bouin's solution.
b - Any uterus stained in 10% ammonium solution for detection of implantation sites will be discarded and will not be preserved in 10% neutral buffered formalin.
c - Representative sections of corresponding organs from a sufficient number of controls will be retained for comparison, if possible.

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8.2 Organ Weights:

The following organs will be weighed from all F_0 parental animals euthanized at scheduled termination. Organ-to-final-body weight and organ-to-brain weight ratios will be evaluated.

Brain	Ovaries (with oviducts)
Epididymides*	Pituitary
Kidneys	Testes*
Liver	

* - These paired organs will be weighed separately.

8.3 Microscopic Examination:

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the following tissues from all F_0 parental animals from the control and high-dose groups and from all parental animals dying spontaneously or euthanized *in extremis*. If a target organ is identified in the high-dose group, this organ will be examined from all animals in the low and mid-dose groups (at additional cost):

Cervix	Seminal vesicles
Coagulating gland	Testes
Epididymides	Uterus
Ovaries and oviduct	Vagina
Prostate	All gross (internal) lesions

The slides will be prepared by WIL Research Laboratories, LLC and then shipped to Sponsor at the address and contact below for examination by the Principal Investigator, Pathology.

Carolyn Lloyd
DuPont Haskell Global Centers for Health & Environmental Sciences
Investigative Sciences, S320/531
1090 Elkton Road
Newark, DE 19714-0050
Tel: 302-366-5401
Fax: 302-451-4530
Email: carolyn.w.lloyd@usa.dupont.com

The examination of the slides will be performed by the Principal Investigator for Pathology. A final pathology report will be prepared and submitted to WIL Research for inclusion as an appendix in the main study final report. A Quality Assurance and GLP compliance statement signed by the performing laboratory

will be provided to the WIL Study Director for inclusion in the Final Report. The Sponsor is responsible for archiving of raw data associated with the conduct of the pathological examination.

9 DURATION OF STUDY:

The two generations to be studied (parental animals and first generation offspring) will be termed F_0 and F_1, respectively. The conduct of this study will require approximately 22 weeks for acclimation, mating, gestation and lactation of the F_0 generation.

10 STATISTICAL METHODS:

All analyses will be two-tailed for significance levels of 5% and 1%. All means will be presented with standard deviations. All statistical tests will be performed by a computer with appropriate programming as referenced below. The litter, rather than the pup, will be considered as the experimental unit.

10.1 Parental In-Life Data:

Continuous data variables [mean body weights, body weight gains and food consumption at each interval], pre-coital intervals, gestation length, former implantation sites, unaccounted-for sites, mean days of attainment of developmental landmarks (balanopreputial separation and vaginal patency) and the body weight on the day of attainment will be subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor, 1980) to determine intergroup difference. If the results of the ANOVA are significant ($p<0.05$), Dunnett's test (Dunnett, 1964) will be applied to the data to compare the treated groups to the control group.

Male and female mating, fertility, copulation and conception indices of the treated groups will be compared to the control group using the Chi-square test with Yates' correction factor (Hollander, 1999).

10.2 Litter Data:

The mean litter proportions (% per litter) of pup viability during the postnatal period and sex ratio at birth will be subjected to the Kruskal-Wallis nonparametric ANOVA test (Kruskal, 1952) to determine intergroup difference. If the results of the ANOVA are significant ($p<0.05$), the Dunn's Test (Dunn, 1964) will be applied to compare the treated groups to the control group. Mean numbers of pups born, live litter size and litter weights will be subjected to the parametric ANOVA test (Snedecor, 1980) and Dunnett's test (Dunnett, 1964) as described above with the litter representing the experimental unit.

10.3 Histopathology and Organ Weight Data:

Histopathological findings of each treated group will be compared to those of the control group by the Fisher's Exact test (Steel, 1980). Organ weights (absolute and relative to body weights and relative to brain weights) will be subjected to a parametric ANOVA test (Snedecor, 1980) and Dunnett's test (1964) as described above.

11 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with the study protocol and protocol amendments, WIL Standard Operating Procedures and the appropriate provisions of EPA/TSCA and FIFRA Good Laboratory Practice Standards published in the Federal Register (40 CFR Part 792 and 40 CFR Part 160) and the OECD Principles of Good Laboratory Practice. The final report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor Representative to assure that the final report accurately describes the conduct and the findings of the study.

The pathological examination of the slides will be conducted following the Standard Operating Procedures of the performing laboratory and in accordance with GLPs. Quality Assurance monitoring of these analyses for SOP and GLP compliance is the responsibility of the performing laboratory. Inspection reports will be supplied to the Study Director. Upon completion of the prescribed activities and submission of the results to the Sponsor and Study Director the performing laboratory will provide a signed Quality Assurance Statement to the Sponsor (copy to the Study Director). The results will be included in the final report.

This study will be included on the WIL master list of regulated studies.

12 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored as described in Section 13 in the Archives at WIL Research Laboratories, LLC.

The Sponsor will be responsible for the archival of the raw data and records for the pathological examination.

13 WORK PRODUCT:

The Sponsor will have title to all documentation records, raw data, slides, specimens and other work product generated during the performance of the study. Any remaining formulation samples will be discarded after the issuance of the Final Report. All work product, including raw paper data, pertinent electronic storage

media and specimens, will be retained for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

All work product generated at a performing laboratory will be retained at an appropriate archive facility as designated by the SOPs of the performing laboratory.

14 REPORTS:

The final report will contain a summary, test item data, methods and procedures, maternal and pup data WIL Historical Control Data, the analytical chemistry report, pathology report and an interpretation and discussion of the study results. The final report will be comprehensive and shall define level(s) inducing toxic effects as well as no-effect level(s) under the conditions of this investigation. The report will contain all information necessary to conform with current OPPTS and OECD specifications.

WIL Research Laboratories, LLC will submit one copy of an audited draft report in a timely manner upon completion of data collection prior to issuance of the final report. One revision will be permitted as part of the cost of the study, from which the Sponsor's reasonable revisions and suggestions will be incorporated into the final report, as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL Research Laboratories, LLC within a two-month time frame following submission. WIL Research Laboratories, LLC will submit the final report within one month following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL Research Laboratories, LLC within one year following submission of the draft report, WIL Research Laboratories, LLC may elect to finalize the report following appropriate written notification to the Sponsor. Two electronic copies (PDF) of the final report on CD-R will be provided. Requests for paper copies of the final report may result in additional charges.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act (AWA) regulations (9 CFR Parts 1, 2 and 3). The Sponsor should make particular note of the following:

- The Sponsor Representative's signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.

- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.

- Animals that experience severe pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.

- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of protocol amendment. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

17 REFERENCES:

Adams, J.; Buelke-Sam, J.; Kimmel, C.A.; Nelson, C.J.; Reiter, L.W.; Sobotka, T.J.; Tilson, H.A.; Nelson, B.K. Collaborative behavioral teratology study: protocol design and testing procedure. *Neurobehavioral Toxicology and Teratology* 1985, *7*, 579-586.

Dawson, A.B. A note on the staining of the skeleton of cleared specimens with Alizarin Red S. *Stain Technology* 1926, *1*, 123-124.

Dunn, O.J. Multiple comparisons using rank sums. *Technometrics* 1964, *6(3)*, 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* 1964, *20*, 482-491

Haas, M. A 90-Day Oral (Gavage) Study of H-28548 in Rats with a 28-Day Recovery. WIL-189216, Draft.

Hollander, M.; Wolfe, D.A. *Nonparametric Statistical Methods*, 2nd ed.; Hollander, M., Wolfe, D.A., Eds.; John Wiley and Sons, Inc.: New York, NY, 1999; p 468.

Korenbrot, C.C.; Huhtaniemi, I.T.; Weiner, R.W. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction* 1977, *17*, 298-303.

Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* 1952, *47*, 583-621.

Salewski, E. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. [Staining method for a macroscopic test for implantation sites in the uterus of the rat]. *Naunyn - Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie* 1964, *247*, 367.

Snedecor, G.W.; Cochran, W.G. One Way Classifications; Analysis of Variance. In *Statistical Methods*, 7th ed.; The Iowa State University Press: Ames, IA, 1980; pp 215-237.

Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics, A Biometrical Approach*, 2nd ed.; McGraw-Hill Book Company: New York, NY, 1980; pp 504-506.

ED_002003A_00070249-00024

Stuckhardt, J.L.; Poppe, S.M. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratogenesis, Carcinogenesis and Mutagenesis* **1984,** *4,* 181-188.

18 PROTOCOL APPROVAL:

Sponsor approval received via email on 4 Jan 2010.
Date

E. I. du Pont de Nemours and Company

Susan M. Munley
Susan M. Munley, MA
Sponsor Representative

8 Jan 2010
Date

WIL Research Laboratories, LLC

Tammye L. Edwards
Tammye L. Edwards, BS, LAT
Study Director

4 Jan 2010
Date

Donald G. Stump
Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

4 Jan 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 1

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) Applicable Protocol Sections: 3

   The proposed audited draft date is 10 September 2010.

2) Applicable Protocol Sections: 7.2

   The control group and three test item groups will consist of 25 males and 25 females each.

3) Applicable Protocol Sections: 7.3

   Appropriately sized flexible, Teflon®-shafted, stainless steel dosing cannulae will be used for the oral administration by gavage. The dosing cannulae may or may not be ball-tipped as appropriate for the age of the animal.

4) Applicable Protocol Sections: 7.4.3.2

   F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 20, inclusively, for females that deliver.

5) Applicable Protocol Sections: 7.6

   Detection of mating will be confirmed by the appearance of a vaginal copulatory plug.

6) Applicable Protocol Sections: 7.9.2.2

For those females with evidence of mating, body weights will be recorded individually on a weekly basis, beginning on the first day of dose administration, until evidence of copulation is observed and on gestation days 0, 4, 7, 11, 14 and 18 and on lactation days 1, 4, 7, 14 and 21.

7) Applicable Protocol Sections: 7.11.1.2

On post-mating day 23 (females with evidence of mating) or post-cohabitation day 23 (females without evidence of copulation), the F_0 females which fail to deliver will be euthanized by carbon dioxide inhalation.

8) Applicable Protocol Sections: 7.12.1

The second sentence of the first paragraph is changed to the following:
A detailed physical examination will be conducted weekly.

9) Applicable Protocol Sections: 7.12.2

F_1 males and females will be have a body weight recorded approximately weekly, beginning with the start of test substance administration until euthanasia (PND 21, 28, 35 and 40).

10) Applicable Protocol Sections: 8.1

Footnote "a" should read:

Testes and epididymides will be fixed in Bouin's solution. Care will be taken to ensure separation between the left and right organs.

11) Applicable Protocol Sections: 8.3

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the listed tissues from all F_0 parental animals from the control and high-dose groups and from all parental animals dying spontaneously or euthanized *in extremis* and from any animals in the low and mid dose groups with impaired fertility (males that did not sire a litter or females that did not deliver a litter).

Reasons for Protocol Modification:

1) Audited report date added to protocol.

2) The number of animals in each dose group was increased to 25 per sex to ensure an adequate number of pregnant females per group.

3) Ball-tipped steel gavage needles are not used on pups under 28 days of age.

4) Clarification of dosing regimen for the females that deliver.

5) Vaginal lavages are not used for the determination of pregnancy in mice, just the presence of copulatory plugs.

6) Correction of gestation days body weights are collected and mice deliver on GD 18.

7) Change in the post-mating or post-cohabitation day that the mice will be euthanized on due to the mouse having a shorter gestation length.

8) F_1 clinical observations were changed to weekly physical examinations for consistency with the F_0 observations.

9) Correction of typographical error.

10) Clarification of maintenance of left and right organ separately for necropsy tissue collection.

11) Addition of microscopic evaluation of animals in the low and mid dose group that have impaired fertility.

Approval:

Sponsor's approval was obtained via email on January 19, 2010.

Tammye L. Edwards, BS, LAT
Study Director

22 Jan 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

22 Jan 2010
Date

Susan M. Munley, MA
Sponsor Representative

29 Jan 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 2

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **5.6 Approximate Age:**

   The approximate age of the males at randomization will be 42-63 days. The approximate age of the females at randomization will be 70-80 days.

2) **6.1 Animal Housing:**

   The females will be housed individually in solid bottom cages upon arrival.

Reasons for Protocol Modification:

1) Age of mice changed to ensure sexual maturity at the time of breeding.

2) The caging upon arrival was changed to individual due to the increase in age of the animal upon arrival.

Approval:

Sponsor's approval was obtained via email on February 10, 2010.

WIL Research Laboratories, LLC

Tamraye L. Edwards, BS, LAT
Study Director

12 Feb 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

12 Feb 2010
Date

E.I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

15 Feb 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 3

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **7.9.3.2 Females:**

The first paragraph is changed to the following:

Recorded individually on a weekly basis beginning on the first day of dose administration, until the start of the mating period. Individual food consumption will be recorded on the day evidence of copulation is observed (GD 0) and on gestation days 4, 7, 11, 14 and 18 and lactation days 1, 4, 7, 14 and 21.

Reasons for Protocol Modification:

1) Gestation food consumption intervals corrected for the mouse gestational period.

Approval:

Sponsor's approval was obtained via email on March 11, 2010.

WIL Research Laboratories, LLC

Tammye L. Edwards, BS, LAT
Study Director

11 March 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

16 Mar 2010
Date

E.I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

12 March 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 4

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **1 Objective:**

   The following is added to this section:

   In addition, a toxicokinetic assessment of plasma levels of the test article will be performed in the F_0 females and the F_1 pups at culling and on PND 21 and PND 40.

2) **2.4 WIL Departmental Responsibilities:**

   The following person is added to this section:

   Carol S. Wally, BA, SRS, RLATG
   Group Supervisor, Sample Processing Laboratory

3) The following sections are added to the protocol:

   2.5 Principal Investigator, Plasma Sample Analysis and Report:

   Michael Mawn, PhD
   Senior Research Chemist
   DuPont Stine-Haskell Research Center
   1090 Elkton Road
   Bldg. S-315 Lab 1333
   Newark, DE 19714-0030
   Tel: 302-451-3365
   Email: michael.p.mawn@usa.dupont.com

4) The following sections are added to the protocol:

7.15 Plasma Sample Collection and Analysis:

7.15.1 Interval:

Blood samples will be collected at the time of scheduled necropsy on LD 21 from 5 randomly selected F_0 females per group that delivered. A blood sample will be collected from all females that failed to deliver on post-mating day 23 at the time of the scheduled necropsy.

In addition, all control females that delivered but were not selected for blood collection as indicated above, will have blood samples taken on LD 21 at the time of scheduled necropsy to provide control animal plasma for method development work to be conducted by the Sponsor. These control samples will be processed and shipped as described for the study samples.

Blood samples will also be collected from the F_1 culled pups on PND 4 from 10 randomly chosen litters in each group following culling and data collection.

On PND 21, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy that are not selected for the F_1 generation.

On PND 40, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy.

7.15.2 Route of Collection:

Blood samples will be collected via the vena cava following euthanasia by carbon dioxide inhalation from the F_0 females and the F_1 PND 21 and PND 40 animals.

Blood samples will be collected via decapitation from the PND 4 pups and pooled by litter.

7.15.3 Target Blood Volume:

For the F_0 females and the F_1 PND 21 and PND 40 animals, 1.0 mL or as much as possible, will be collected into pre-chilled, uniquely-labeled tubes. For the PND 4 pups, blood will be pooled by litter from all the culled pups in each litter to obtain as much blood as possible.

7.15.4 Anticoagulant:

K_3EDTA

7.15.5 Sample Handling and Plasma Preparation:

Samples will be kept on wet ice, protected from light, until centrifugation. All samples will be centrifuged [approximately 3000 rpm (approximately 2060 x g) for approximately 10 min] at approximately 4°C. Plasma will be transferred into new, uniquely-labeled polypropylene tubes.

7.15.6 Label Information:

Samples will include study number, dose group, animal number, interval, sample type and date and time of blood collection.

7.15.7 Storage:

Plasma samples will be stored frozen at approximately -20°C until analysis. The time and date the samples were placed in the freezer will be recorded.

7.15.8 Sample Shipment:

Frozen samples in dry ice, an inventory list and documentation of actual blood collection times for each animal will be shipped on the first Monday or Tuesday after the last sample is collected. The recipient will be notified at least 24 hours in advance of any shipment. Samples will be shipped overnight to:

Michael Mawn, PhD
Senior Research Chemist
DuPont Stine-Haskell Research Center
1090 Elkton Road
Bldg. S-315 Lab 1334
Newark, DE 19714-0030
Tel: 302-451-3365
Email: michael.p.mawn@usa.dupont.com

7.15.9 Plasma Analyses and Report:

Plasma samples will be analyzed for the test article content after solvent protein precipitation with LC/MS/MS analysis. The method of analysis will be documented in the study records and final report. The Principal Investigator for the plasma analysis will be responsible for all bioanalytical delegated-phase activities and will issue a formal bioanalytical/plasma analyses report from the data generated that will be included as an appendix in the final report. A Quality Assurance and GLP compliance statement signed by Sponsor and archival location of the data will be provided to the WIL Study Director for inclusion in the Final Report.

5) **11 Quality Assurance:**

The first sentence of the second paragraph is changed to the following:

The plasma samples analysis and the pathological examination of the slides will be conducted following the Standard Operating Procedures of the performing laboratory and in accordance with GLPs.

6) **12 Records To Be Maintained:**

The second paragraph is changed to the following:

The Sponsor will be responsible for the archival of the raw data and records for the plasma sample analyses and the pathological examination.

7) **13 Work Product:**

The second sentence of the first paragraph is changed to the following:

Any remaining plasma samples and formulation samples will be discarded after the issuance of the Final Report.

8) **14 Reports:**

The second sentence of the first paragraph is changed to the following:

The final report will contain a summary, test item data, methods and procedures, maternal and pup data WIL Historical Control Data, the analytical chemistry report, the plasma analysis report, the pathology report and an interpretation and discussion of the study results.

Reasons for Protocol Modification:

1-8) Blood collection for plasma sample analyses is added to the protocol at the Sponsor's request to characterize the exposure levels of the test substance.

Approval:

Sponsor's approval was obtained via email on April 14, 2010.

WIL Research Laboratories, LLC

Tammye L. Edwards, BS, LAT
Study Director

15 April 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental and
Reproductive Toxicology

15 Apr 2010
Date

E. I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

19 Apr 2010
Date

Study Number: WIL-189225

PROTOCOL AMENDMENT 5

Sponsor: E.I. du Pont de Nemours and Company

Title of Study:

An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice

Protocol Modifications:

1) **7.4.3.2 Females:**

The first sentence of this section is changed to the following:

F_0 females will be dosed for a minimum of 14 days prior to mating and continuing throughout mating, gestation and lactation until Lactation Day (LD) 20, inclusively, for females that deliver, with the exception of the 5 females/group that are selected for blood collection on LD 21, which will also receive a dose on LD 21.

2) **7.4.3.3 F_1 Males and Females:**

F_1 males and females will be dosed beginning in PND 21 through PND 40, inclusively.

3) **7.15.1 Interval:**

This section is changed to the following:

Blood samples will be collected at 2 hours post dose administration on LD 21 at necropsy from 5 randomly selected F_0 females per group that delivered. A blood sample will be collected from all females that failed to deliver on post-mating day 23 at the time of the scheduled necropsy (not timed).

In addition, all control females that delivered but were not selected for blood collection as indicated above, will have blood samples taken on LD 21 at the time of scheduled necropsy (not timed) to provide control animal plasma for method

development work to be conducted by the Sponsor. These control samples will be processed and shipped as described for the study samples.

Blood samples will also be collected from the F_1 culled pups on PND 4 from 10 randomly chosen litters in each group following culling and data collection.

On PND 21, blood samples will be collected from 5 randomly selected F_1 males and females in each group at the time of the scheduled necropsy (not timed) that are not selected for the F_1 generation.

On PND 40, blood samples will be collected at 2 hours dose administration at necropsy from 5 randomly selected F_1 males and females in each group.

Reasons for Protocol Modification:

1-3)Per the Sponsor, the most appropriate time of blood collection is 2 hours following dose administration; therefore, an additional dose day for the LD 21 females selected for blood collection and an additional dose day for all F_1 pups on PND 40 was added and the time and days of sample collection was added as appropriate.

Approval:

Sponsor's approval was obtained via email on May 3, 2010.

WIL Research Laboratories, LLC

Tammy L. Edwards, BS, LAT
Study Director

4 May 2010
Date

Donald G. Stump, PhD, DABT
Director, Developmental
and Reproductive Toxicology

4 May 2010
Date

E. I. du Pont de Nemours and Company

Susan M. Munley, MA
Sponsor Representative

5 May 2010
Date